QUANTITATIVE ANALYSIS OF ROOT DAMAGE AND REPAIR FOLLOWING PLACEMENT OF MINISCREW USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X-RAY ANALYSIS

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CERTIFICATE

This is to certify that this dissertation titled **"QUANTITATIVE ANALYSIS OF ROOT DAMAGE AND REPAIR FOLLOWING PLACEMENT OF MINISCREW USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X-RAY ANALYSIS."** is a bonafide record of work done by **DR S. VINOTH** under my guidance during his postgraduate study period 2009–2012.

This dissertation is submitted to **THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **Master of Dental Surgery** in Branch V – Orthodontics and Dentofacial Orthopedics

It has not been submitted (partially or fully) for the award of any other degree or diploma.

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CONTENTS

INTRODUCTION

Orthodontic anchorage control is a fundamental and challenging part of orthodontic treatment planning and subsequent treatment delivery. There are times when absolute or maximum anchorage, is needed. However Newton's third law states that an applied force can be divided into an action component and into an equal and opposite reaction component. Therefore, it is virtually impossible to achieve absolute anchorage in which the reactionary force produces no movement of the anchorage segment.

Traditionally extra-oral anchorage has been used to reinforce intraoral anchorage. The use of extra oral anchorage ideally demands co-operation of the patient as well as 24 hours of continuous wear, objectives that are difficult to achieve. Therefore, it is extremely difficult to undertake orthodontic treatment without compromising anchorage in some way. This lead to the development of intraoral extra-dental anchorage such as "Temporary anchorage devices"(TAD's).

Although these devices are available in several sizes and designs, such as miniscrews, mini-implants, microscrew, the most popular and widely used temporary anchorage devices appears to be the miniscrew.[21]

Possible site for miniscrew placement includes the palate, mandibular retro molar area, maxillary tuberosity, anterior nasal spine, mandibular symphysis, and edentulous areas of the alveolar ridge**.** One of the most common locations is the interradicular space between adjacent teeth and

2-Dimentional radiographic studies showed that 2mm safety clearance is recommended between miniscrew and root surface[53] and also interroot distance must be atleast 4mm for placement of miniscrew.[73]

Although several authors have developed and suggested new 3-Dimentional CBCT surgical guides for precise miniscrew placement in interradicular spaces.[58,38,44] These guidelines aid clinicians, but do not take account of individual differences in root morphology. A Recent 3-Dimentional CBCT study by **Chung et al** [19] had showed that 65.7% root damage occurs even if there is adequate amount of interradicular space. Root damage can also occur due to improper placement of miniscrew,[47] due to angulation difference in placement,[43] and due to migration of miniscrew after loading,[53,78] thus root proximity is a major risk factor for failure in orthodontic anchorage while using these devices**.**[48] This may leads to incidence of root resorption and ankylosis of teeth when the damage is severe.[49]

Therefore, Miniscrews should be placed into the alveolar bone without risk of damage to adjacent roots. Since the available space increases from alveolar crest towards apical direction, It was suggested that miniscrews should be placed in the apical region.[67] However, the problem with this might be that screws placed in unattached gingiva can lead to inflammation of periimplant soft tissue which may lead to implant failure.[58] Therefore placement in the attached gingiva or on the border between attached and unattached gingiva is preferable.

Asscherickx et al [4] conducted experiments on beagle dogs and reported that healing takes place in approximately 12 weeks after root damage with miniscrew, and healing was nearly completed after 20 weeks. In a classical animal study, **Chen et al**[17] found higher failure rates when miniscrew contacted the root, they also reported repair of roots by cementum deposition and bone regeneration when the miniscrews were removed and the roots were allowed to heal. Even though animal studies have been reported in literature on this regard, the results of these studies cannot be extrapolated to the human beings.

A case series by **Kadioglu et al** [41] in human samples by using scanning electron microscope, indicates that root surfaces contacted with miniscrews showed swift repair and healing progressed within a few weeks after removal of the miniscrew, but these studies are with insufficient sample size and didn't report of any change in composition of root and periodontium during repair period.

Composition of periodontium differs with respect to location, structure, function, rate of formation, and degree of mineralization.[20] **Rex et al** [72] reported there is an increasing gradient in the calcium and phosphorus concentration from the outer to the inner third of cementum at the cervical and middle third of the root. **Cohen et al** [20] found that when diseased cementum is exposed to the oral cavity it showed an increase in calcium and phosphorus content at a depth of 40µm. A few studies have used an electron microprobe to analyse the distribution of various elements in cementum, [7,20,61,72,74] but no consensus could be reached regarding the occurrence or, distribution of various elements. .

There is scant evidence regarding the histomorphometric and scanning electron microscopic features of root during repair process that occurs after damage with miniscrew. In order to understand the nature of cementum during its repair process, the knowledge of the various elemental contents of normal and healing root surface is required. [42]

To our knowledge there is no study that assesses the compositional changes of damaged cementum during repair process.

There are few quantitative studies on the chemical composition of cementum by using chemical analysis and micro radiographic techniques. One of the major problems in chemical analysis is that it is difficult to obtain large enough samples, and require the separation of cementum from other tissues which leads to destruction of samples and altered the elemental content [61] and microradiography is limited because it usually yields data only on calcium composition.[2,8]

Therefore using the scanning electron microscope with energy dispersive X-ray detector attachment for analysis of the chemical composition of cementum which can yield data on small samples of tissue in situ and in a non-destructive manner with high level sensitivity was considered. Further it also eliminates the sampling problems associated with other technique [42] and unlike microradiography, the composition of other elements in addition to calcium can also be determined.

Therefore, the aim of our study was to examine the clinical consequences of injury to the root surfaces when miniscrews were intentionally placed into direct contact with premolar roots for defined periods in an experimental human model. The quantitative changes in the periodontal ligament, cementum, and dentin during these periods of contact and repair were examined by using scanning electron microscopy (SEM) and compositional changes were analysed by using Energy Dispersive X ray microanalysis (EDX).

Review of Literature

Introduction

Tables & Graphs

REVIEW OF LITERATURE

Literature has been reviewed under the following headings:

- Osseo Integrated Implants to miniscrew implants in orthodontics
- Grids for precise positioning of mini screws
- Iatrogenic injuries during mini screw placement
- Studies on tooth reparative potential for root resorption
- Compositional changes of Periodontium.

Skeletal anchorage has evolved as a mainstream in orthodontic anchorage in the past decade. Dental implants, mini plates and titanium screws have been used as skeletal anchorage, because these devices can provide absolute anchorage without patient cooperation. Titanium screws are currently in vogue because the screws are useful for various orthodontic tooth movements with minimal anatomic limitation as placement is easy with less trauma and less cost. Mini implant anchorage or skeletal anchorage system is becoming an increasingly significant part of orthodontic treatment and has recently gained interest as anchorage units for orthodontic purposes.

Osseo Integrated Implants to miniscrew implants in orthodontics:

Dahl [40](1945) first published the use of the sub periosteal vitallium implants to effect tooth movement in dogs.

Linkow [40](1966) described endosseous blade implants with perforations for orthodontic anchorage.

Branemark [40](1969) the mentor of modern implant surgery described a high compatibility and strong anchorage of titanium in human tissue and coined the term "Osseointegration".

Kawahara et al [40](1975) developed Bioglass coated ceramic implants for orthodontic anchorage.

Branemark et al [40](1977) defined Osseointegration as a direct structural and functional connection between living bone and the surface of a load carrying implant.

Creekmore [40](1983) reported the possibility of skeletal anchorage in orthodontics.

Roberts[40] (1989) used conventional, two stage titanium implants in the retro molar region to help reinforce anchorage. After completion of the orthodontic treatment the implants were removed using a trephine and were histologically analyzed. He found a high level of Osseointegration despite the orthodontic loading.

Higuchi and James[40] **(1991)** used titanium fixtures for intra oral anchorage to facilitate orthodontic tooth movement.

Costa et al [40]**(1998)** used mini screws for orthodontic anchorage.

Umemori et al[40] **(1999)** used Skeletal Anchorage System for open bite correction.

Liou[53] **(2004)** stated that miniscrews are a stable anchorage but do not remain absolutely stationary throughout orthodontic loading. To prevent any contact between miniscrews and vital structures because of displacement, it is recommended that they be placed in a non–tooth-bearing area that has no foramen, major nerves, or blood vessel, or in a tooth-bearing area with 2 mm of clearance between the mini screw and root.

<u>Grids for precise positioning of mini screws</u>

Morea C[60] **(2005)** described a new surgical guide that provides three dimensional control for accurate placement of self-tapping orthodontic mini implants at the desired location and angle. A cast is made of appropriate dental arch. The pre surgical radiographs are evaluated with the parallax technique to assess the interproximal bone morphology and root proximity. Then 2mm hollow steel sleeves with an internal diameter of 1.3mm are cut and affixed to the plaster cast with wax at the proper implant locations and angles. A 1.2mm diameter surgical bur is inserted into the sleeve to assist in correctly orienting the sleeve to the desired angulation. The cast is waxed around the implant holes and occlusal areas, and an acrylic transfer tray is fabricated. After polymerization the surgical guide is trimmed to its final size and shape. The guide is submerged in 1% chlorhexidine solution for 12hrs prior to implant placement. At the first appointment surgical guide is tried in the mouth and pilot holes drilled in the implant site through the metal sleeve of the surgical guide.

R. R. J. Cousley [68] (2005) described the key aspects of stent fabrication in order to simplify the surgical stage and provide optimal insertion of Orthosystem palatal implants. Once the implant's optimal anteroposterior position and inclination have been determined radiographically, then this information is transferred to a working model of the maxillary dentition and palate. A 1.6 mm hole is drilled, using a vertical pillar drill, in the center of the premolar teeth identified in the radiographic plan. The model is radiographed in a cephalostat by placing it on a horizontal platform within the machine. This produces a lateral cephalograph showing the provisional implant location and inclination relative to the vertical plane. The crucial component of the surgical stent is its central guide channel, which dictates the prescribed insertion site and inclination (in both sagittal and transverse planes) by providing three-dimensional control for the surgical instruments. After trimming the base plate edges, an acrylic block is built up between the canines and first molars to approximately 1 cm depth. The model is reseated on the angled table set at then prescribed implant inclination and positioned under the vertical pillar drill. A 3 mm pilot drill is used to make a cylindrical channel through both the central block and base plate. The pilot channel is enlarged by the use of a drill bit, which corresponds to the 5.6 mm (7/32 inch) mid shaft diameter of the implant profile drill. The stent is removed from the model and acrylic relieved from along the anterior aspect of the cylindrical channel to convert it into an approximately one-third open cylinder. The channel is also trimmed around the fitting surface. These modifications provide open access anteriorly for an unobstructed view of the drill and for its direct irrigation, especially at the insertion point. The cephalometric planning described here enables the orthodontist to determine the optimal anterioposterior position and inclination for palatal implants.

Sergio Estelita[75] **(2006)** described a three dimensional radiographic surgical guide (RSG) that ensured the exact correspondence between x-ray and drill trajectories. In this radiographic – surgical guide (RSG) with an .045 stainless steel telescopic tube soldered to the end of a vertical arm ,which is attached to a horizontal arm by a gurin lock. Both arms are made of .021× .025 stainless steel wire allowing the RSG to be inserted into the fixed appliance. The modified radiographic positioner (MRP) is a bite wing positioner with a securely attached .040 stainless steel wire. The free end of this wire, perpendicular to the film plane, is inserted into the telescopic tube of the RSG. As a radiographic guide, the RSG positions the MRP, which defines x-ray path. If the pre surgical radiograph shows the telescopic tube in a safe relationship to the inter radicular septum and adjacent roots, the RSG can also be used as a guide for the pilot drill. The tube should be long enough to allow the drill to penetrate the mucosa and bone tissue of inter radicular septum to a depth corresponding to the selected mini implant length.

Poggio [67](2006) provided an anatomical map to assist the clinician in miniscrew placement in a safe location between dental roots. For each interradicular space, the mesiodistal and the buccolingual distances were measured at two, five, eight, and eleven mm from the alveolar crest. In the maxilla, the greatest amount of mesiodistal bone was on the palatal side between the second premolar and the first molar. The least amount of bone was in the tuberosity. The greatest thickness of bone in the buccopalatal dimension was between the first and second molars, whereas the least was found in the tuberosity. In the mandible, the greatest amount of mesiodistal dimension was between first and second premolar. The least amount of bone was between the first premolar and the canine. In the buccolingual dimension, the greatest thickness was between first and second molars. The least amount of bone was between first premolar and the canine. He concluded that “safe zone” of screws with a diameter of 1.5 mm need at least 3.5 mm of interradicular space to prevent root damage.

Choi HJ[18] (2007) evolved a precise wire guide for positioning interradicular mini screws to avoid root damage and improve the insertion success rates. The wire guide consist of two parts a positioning gauge attached to the tooth distal to the mini screw placement site, and a directional guide attached to the tooth mesial to the mini screw. Weld five to seven 2-3 mm lengths of .014 Elgiloy make a bayonet bent at the mesial opening of the first molar buccal tube to act as a stop taking care not to contact the second molar bracket. After fitting the two parts of the wire guide in the mouth take another periapical radiograph to show the position of the roots in relation to the stent. The occlusal arm should be perpendicular to the lower border of the x-ray film, so that the x-ray beam is parallel to the occlusal arm of the directional guide. On the radiograph determine the midpoint between the two adjacent roots based on the wire segments welded to the horizontal arm of the positioning gauge. Using a mirror to visualize the occlusal surface, insert the mini screw 2-3 mm to the occlusal arm of the directional guide.

Kravitz[46] (2007) described a simplified stent that can be fabricated at the chairside. Clinically locate the roots adjacent to the miniscrew insertion site by firmly pressing the long end of a periodontal probe against the buccal tissue. Securely tie two L-shaped rectangular wires, facing each other, into the bracket slots adjacent to the miniscrew site. These wires should extend vertically well beyond the mucogingival junction, following the outer surfaces of the roots, and horizontally past the outer edges of the brackets. Take a periapical radiograph to confirm the proper positioning of the stent. With periodontal probe, press firmly against the tissue at the exact site of insertion. This soft-tissue “punch” provides a visual marker and helps prevent slippage during self-drilling of the miniscrew. This stent can also be used for placement of posterior interradicular miniscrews.

Bharani Kumar Reddy [9] (2008) described a grid for mini screw placement. It is fabricated from .016 × .022 stainless steel wire. The wire is cut and welded to form a three column grid. In which each square measures about 1mm. The appropriate length of the grid is determined by the desired screw insertion point. The grid is attached to a locking device made from an electrical terminal connector with a screw at either end; such a connector can easily be removed from a push button switch or similar component, available in electrical stores. Clinically once the mini screw placement area has been determined peri apical radiograph can be taken with the grid. The appropriate cell of the grid for the exact mini screw site is selected from the radiograph.

Cha BK[16] (2008) evaluated area and gender-related differences in the soft tissue thickness of potential areas for installing miniscrews in the buccal-attached gingiva and the palatal masticatory mucosa. An ultrasonic gingival thickness meter was used to measure the soft-tissue thickness in the buccal-attached gingiva just adjacent to the mucogingival junction of the upper and lower arches and 4 mm and 8 mm below the gingival crest in the palatal masticatory mucosa. Buccal-attached gingiva thickness in the upper arch was significantly greater in men than in women, but buccal-attached gingiva thickness in the lower arch and palatal masticatory mucosa thickness 4 and 8 mm below the gingival crest did not show gender differences. In the palatal masticatory mucosa, significantly thicker soft tissue was found 4 mm below the gingival crest in the anterior areas and 8 mm below the gingival crest in the posterior areas. The areas between the canines and the premolars showed higher values than other areas 4 mm below the gingival crest. However, the soft-tissue thickness 8 mm below the gingival crest showed a progressive increase from the anterior to the posterior areas. He concluded that measurements of the soft-tissue thickness using an ultrasonic device could help practitioners select the proper orthodontic miniscrew in daily clinical practice.

Chung How Kau [19](2010) conducted a study to evaluate the location of TADs placed during orthodontic treatment and to relate the placement to the surrounding dentoalveolar structures Thirty-five TADs (19 in the maxilla, 16 in the mandible) were evaluated. Three-dimensional cone-beam computed tomography scans were taken before and after placement of the TADs over a 6-month period as part of routine clinical protocol. Three-dimensional cone-beam computed tomography technology allows better visualization of TAD placement. Of the 35 TADs, 65.7% were in contact with the periodontal ligament. There appears to be more space for TAD placement in the mandible than in the maxilla.

Hong Liu[38] (2010) was to enable accurate miniscrew placement after preoperative simulation. Eleven patients who had bimaxillary protrusion were scanned with computed tomography. The 3-Dimensional computed tomography data were used to produce, with stereolithography apparatus, a template for accurate miniscrew placement. The interradicular space available for miniscrew placement was calculated in the 3-dimensional images. Postoperative computed tomography images were matched with preoperative images to calculate the deviations between the planned and actual placements and in results showed that distance for placement of a miniscrew between two roots was 4.12 mm (SD, 0.25 mm; range, 3.7-4.5 mm). The placed miniscrews showed an average angular deviation of 1.2° (SD, 0.43°; range, 0.6°-2.41°) compared with the plan, whereas the mean linear distomesial deviation was 0.42 mm (SD, 0.13 mm; range, 0.15-0.6 mm) at the tip and in conclusion proposed template has high accuracy and will be especially useful for patients who require precise miniscrew placement.

Miyazawa Ken[59] (2010) To avoid possible root damage, a surgical guide was fabricated and cone-beam computed tomography (CBCT) was used to incorporate guide tubes drilled in accordance with the planned direction of the implants. Forty-four self-drilling miniscrew implants (diameter 1.6, and length 8 mm) were placed in interradicular bone using a surgical guide procedure, the majority in the maxillary molar area. And he concluded that CBCT images of post-surgical self-drilling miniscrew implant placement showed no root contact (0/44). However, based on CBCT evaluation, it was necessary to change the location or angle of 52.3 per cent (23/44) of the guide tubes prior to surgery in order to obtain optimal placement. The total success rate of all miniscrews was 90.9 per cent (40/44).Orthodontic self-drilling miniscrew implants must be inserted carefully, particularly in the case of blind placement, since even guide tubes made on casts frequently require repositioning to avoid the roots of the teeth. The use of surgical guides, fabricated using CBCT images, appears to be a promising technique for placement of orthodontic self-drilling miniscrew implants adjacent to the dental roots and maxillary.

Iatrogenic injuries during mini screw placement

Fabbroni G [29] (2004) did a prospective study on transalveolar screws and the incidence of dental damage. Patients with fractures of the mandible requiring intra- or post-operative control of their occlusion with transalveolar screws were entered into the study. Following screw removal, contact was assessed radiographically and all teeth adjacent to screws were tested for vitality. Any contacts were judged to be minor if less than 50% of the diameter of the screw hole impinged on a tooth root or major if it was more than 50%. There were 232 screws placed in these patients adjacent to 440 teeth. Twenty-six screws (11.2%) had major contacts and 37 (15.9%) had minor contacts. Seventeen teeth tested as non-vital with electronic pulp testing but of these only 6 showed any impingement by screws. Two screws were associated with complications in two patients. Screw/tooth contact does occur using transalveolar screws; however the incidence of clinically significant damage appears to be very low.

Driemel O [26] (2005) did a study on dental injuries due to miniplate osteosynthesis. During a period of 11 years, 380 patients with permanent dentition underwent miniplate osteosynthesis for the treatment of mandibular fractures. These patients were clinically and radiographically examined for a follow-up time of not less than 38 months. The 29 patients could be classified into four different types of dental root trauma: 13 pulp injuries above the apical third of the root (type Ia), 6 pulp injuries in the apical third of the root or extradental lesions interrupting the apical blood stream (type Ib), 4 lesions to the central radicular dentin without pulp injury (type II), and 6 lesions to the peripheral radicular dentin and root cementum (type III). Of 13 type Ia injuries, 5 developed apical periodontitis and dilatation of the periodontal space. One tooth had to be extracted. Three further type Ia injuries and two type Ib injuries showed root resorptions inducing two root canal treatments. One of six type Ib injuries required root canal treatment because of apical periodontitis. One of four type II injuries caused root resorption not requiring therapy. No relevant, pathological finding could be identified after type III injury. The type of dental root trauma caused by miniplate osteosynthesis determines the kind of therapy, complication rate, and survival of the injured tooth.

Asscherickx K.[4] (2005) did an animal - experimental study by placing twenty mini - screws (bracket screw bone anchors, BSBAs) into the mandible of five beagle dogs. Each dog received two bracket screw bone anchors in each lower quadrant, between the roots of the second and third, and third and fourth premolars. Sequential point labelling was performed every 6 weeks with vital stains, and apical x-rays were taken every 6 weeks. Radiographic examination demonstrated damage happend at three roots because of insertion of bracket screw bone anchors. Histological examination of these three roots demonstrated an almost complete repair of the periodontal structure (eg. Cementum, periodontal ligament and bone) in a period of 12 weeks following the placement of the screws.

Maino B. Giuliano [54] **(2007)** investigated the effects of contact between a drill, a mini screw, or both and the roots of four upper premolars in two adolescent orthodontic patients by means of histological analysis. Orthodontic mini screws* (1.5mm in diameter, 8mm long) inserted adjacent to the four upper first premolars, three mesially and one distally. In the three mesial mini screw sites, a 150g super elastic open-coil spring was placed between the second and first premolars to move the roots of the first premolars against the screws. In the distal mini screw site, an open-coil spring was placed between the canine and the first premolar to move the premolar distally against the screw. The open-coil springs were removed after three months of contact between the screws and roots on the patient’s right sides, but left in place on the patients left sides for an additional two months. Next to simulate root damage from a pilot drill; a notch was made with a bur on the distal root surface of each of the three premolars with mesial mini screws, and on the mesial root surface of the premolar with the distal mini screw. These two miniscrews were removed seven days later; after a 30-day healing period, the teeth were extracted under local anesthesia. Histological examination showed active resorption lacunae without signs of repair in the teeth whose roots were pushed against the screws until extraction. In the teeth where contact with the screw had been discontinued before extraction, however, cellular cementum had been deposited, almost entirely filling the resorption craters within two months after removal of the force. In a site where the root was damaged by the pilot drill and subsequent miniscrew insertion, the original contour of the resorption area was evident, as well as incomplete repair of the resorption lacunae with cellular cementum. Clear signs of inflammatory cells could be seen in the periodontal ligament. Similar results occurred when the root was contacted by the pilot drill, but no screw was inserted.

Asscherickx K.[5] (2008) evaluated histologically root contact, proximity to a root, and proximity to marginal bone level as possible risk factors for the failure of mini-screws when inserted between neighboring teeth. Twenty mini-screws were inserted into the mandible of five beagle dogs. Each dog received two bracket screw bone anchors in each lower quadrant, between the roots of the second and third, and third and fourth premolars. Twenty-five weeks after insertion of the screws, the dogs were sacrificed and specimens prepared for histological evaluation. The distance between the screw and the roots and between the screw and the marginal ridge level (MRL) were measured on the histological slides. The presence or absence of root contact was evaluated histologically on serial sections. During the evaluation period, 11 screws were lost. Six screws were in contact with a tooth root and five of these were lost. In five sites, the distance between the screw and the tooth was less than 1.0 mm, but only one of these screws was lost. The distance between the screw and the marginal bone level was less than 1.0 mm for nine screws and seven of these were lost. The results of this limited study suggest that root contact and marginal position might be major risk factors for screw failure.

Chen Yuan- Hou[17] (2008) evaluated the potential problems of damaging adjacent roots and their consequences during mini-implant placement in the alveolar process. Animal experiments were used to evaluate the stability of mini screws placed with intentional root contact. Seventy-two miniscrews were surgically placed in the mandibular alveolar bone of six adult mongrel dogs with metabolic bone labeling at 3-week intervals. Results showed that the miniscrews contacting the roots showed a significantly higher insertion torque than those without contact; and there was a significant difference in the removal torque measurements based on the mobility of miniscrews and the state of root contact; and miniscrews contacting the root were at greater risk of failure. He concluded that during placement of miniscrews in the aveolar process, increased failure rates were noticed among those contacting adjacent roots. When more inflammation was present, the adjacent roots seemed to experience more resorption. Nevertheless, the created lesion was repaired with a narrow zone of mineralized tissue deposited on the root surface, which was likely cellular cementum, and was mainly filled with alveolar bone, with the periodontal ligament space being maintained.

Kadioglu Onur[41] (2008) did a clinical study to examine premolar root surfaces after intentional contact with miniscrews. Ten patients (5 male, 5 female; mean age, 15.8 years; range, 13.5-23.2 years) with 2 maxillary first premolars to be extracted as part of their orthodontic treatment participated in the study. Two miniscrews were placed in each patient, and the first premolar roots were tipped into contact with the miniscrews by using tipping springs with a standardized force. Half of the experimental teeth were kept in contact with the screws for 4 weeks (mild resorption) and the other half for 8 weeks (severe resorption). In 5 patients, the screws were removed, and in the remaining 5 the springs were removed to allow the roots to move back. The roots were allowed to recover for 4 or 8 weeks before extraction. Two premolars with accidental direct contact were used as controls. All teeth were prepared, coated, and examined with scanning electron microscopy. In the control group, the periodontal ligament was removed and the dentin surface denuded. The experimental groups showed signs of resorption with structural surface irregularities. However, no apparent denuded dentin surfaces were seen. Although some resorption lacunae were still discernible at 8 weeks, the collagen fibers fully covered the affected areas. The immature fiber organization in the deepest crater represented the ongoing process of fiber reorganization, compared with the fully matured surface areas surrounding the crater. The results indicate that root surfaces that touch miniscrews show swift repair and almost complete healing within a few weeks after removal of the screw or the orthodontic force.

Hembree M [36] (2009) evaluated the immediate damage to roots and periodontal structures after initial miniscrew implant (MSI) placement and the short and long-term damage after MSIs were left in situ. The roots of the maxillary second, third, and fourth premolars of 7 mature beagle dogs were randomly assigned to undergo immediate, short-term (left for 6 weeks), or long-term (left for 12 weeks) damage. Intentional damage was inflicted with self-tapping screws (1.8 x 8 mm) placed with a stent. Histological sections showed damage to 73.8% of the teeth, ranging from displacement of bone into the periodontal ligament to invasion of the pulp chamber. Displacement of bone into the periodontal ligament and direct damage to the periodontal ligament occurred in 3 (7.2%) instances. Damage was isolated to the cementum of 8 (19.0%) teeth, whereas damage occurred in the dentin of 11 (26.2%) teeth. Loss of bone in the furcation was evident in 3 (7.2%) teeth, and severe damage into the pulp occurred in 6 (14.2%) teeth. No differences in the amounts of damage were evident between the immediate, short and long-term groups. Healing often occurred with cementum around the unloaded MSIs. He concluded that extensive damage can be caused by MSIs, with little to no differences evident over time. Unloaded MSIs that remain in contact with roots of teeth can show varying degrees of healing.

Brisceno Carmen E.[11] (2009) evaluated the healing potential of the roots and surrounding periodontium (cementum, periodontal ligament [PDL], and bone) after intentional damage during mini screw (MSI) placement. Seven skeletally mature male beagle dogs had MSIs placed into the roots of 8 mandibular teeth (6 premolars, 2 first molars). Demineralized and undemineralized sections were stained, and healing was histologically evaluated. The placement torque was twice as high with root contact than without contact (23.8 vs 50.7 Ncm). Damage to the roots and periodontium ranged from cementum interruption to pulp invasion. New bone, PDL, and cementum were observed in 64.3% of the teeth, with significant ($P < 0.05$) increases in the percentages of cementum over time. Sequential labeling confirmed healing at both 6 and 12 weeks. Abnormal healing was found in 35.7% of teeth; it included lack of PDL and bone regeneration, bone degeneration in the furcation area, ankylosis, and no healing associated with inflammatory infiltrate or pulpal invasion. He concluded that under favorable conditions (no inflammatory infiltrate or pulpal invasion), healing can occur when root damage caused by MSIs is limited to the cementum or the dentin. Increased resistance should be used as an indicator of possible root contact during MSI placement.

Yoon-Goo Kang [80] (2009) examined the stability of mini-screws that invade a dental root by measuring the retention period/failure rate, and to illustrate their effects on paradental tissues. Three adult male beagle dogs received 48 orthodontic mini-screws. Half of the mini-screws were implanted to invade the roots, and the rest were placed in the middle of the alveolar bone. Half of the mini-screws were loaded immediately. The failure rate of the mini-screws that invaded the roots was 79.2%, and that of the mini-screws in the middle of the alveolar bone was 8.3%. The application of force had little effect on the failed mini-screws. Moderately injured roots were repaired with osteoid and/or cementoid tissues with normal periodontal ligaments, followed by recovery of the original configuration. He concluded that Orthodontic mini-screws had a higher failure rate when it invade the dental roots. However, minimally damaged dental roots do not adversely affect the healing process.

Renjen Rahul [71](2009) evaluated the histologic response of the dental attachment apparatus and pulp when miniscrews were intentionally placed in contact with or in roots. Sixty self-drilling and self-tapping miniscrews were placed between the premolars and molars of 3 beagles (20 miniscrews per dog). The animals were killed at 12 weeks, and 20 of the most probable injury sites were selected for histologic analysis. The results indicated that there was no histologic evidence of inflammatory response either at the root surface or in the pulp. Pulp necrosis, external resorption, and ankylosis were not found, but reparative cementum was seen at each injury site. He concluded that permanent damage to the pulp and supporting tissues is not a regular occurrence when miniscrews abrade or even enter the root surface.

Kim SH [43](2010), study were to determine factors favouring successful mini-implant placement and to evaluate root proximity as a possible risk factor for failure of osseointegration-based mini-implants during orthodontic treatment Three-dimensional cone-beam computed tomography images were used to examine 50 sandblasted, large-grit, and acid-etched surface-treated mini-implants placed in 25 patients. The images were analyzed for 3-Dimensional position of the mini-implant (placement angle and depth) and any contact with root surfaces or maxillary sinuses. He concluded that Several roots in proximity to the mini-implant combined with sinus perforation without initial stability was defined as the major risk factor for screw failure. The amount of root contact area of a mini-implant is more important for its stability.

Lee YK [49] **(2010)** determines the histological reaction of the root and bone as a mini-implant approaches the root. Two kind of mini-implant were inserted into the buccal alveolar bone of 4 beagles dogs result shows that the incidence of root resorption increased when the mini implant was less than 0.6mm from the root .Root cracking and root fracture occurred in root contact group and root perforation group. Bone resorption and ankylosis were observed in some specimens on the side opposite the insertion and concluded that there is risk of root contact and severe tissue damage from a thick mini-implant and the drilling procedure, either induce root resorption or ankylosis.

Studies on tooth reparative potential for root resorption

Brita Ohm Linge[12] (1983) studied radiographically the incidence and extent of apical root resorption in maxillary incisors in 719 consecutively treated orthodontic patients. Root lengths were measured to the nearest 1/10 mm on standardized intra-oral radiographs taken before and after treatment. Mean root shortening for the four incisors were 0.73 mm and 0.67 mm for girls and boys respectively. When using the most severe single root resorption per patient as a parameter the mean was 1.34 mm for both sexes. A statistical search for clinical risk factors in apical root resorption was done on patients starting treatment after 11 years of age experienced significantly more root resorption than patients starting earlier, even when taking residual root growth into account. Highly significant risk factors were: previous trauma, the correction of impacted maxillary canines, the use of rectangular archwires and Class II elastics. Fixed appliances caused significantly more apical root resorption than removable appliances.

Dermaut L. R. [25](1986) investigated whether root resorption of the upper incisors occurs during intrusion of maxillary incisors. The ratio of root length before and after intrusion was compared in 20 patients. In 66 incisors with an intrusion period of 29 weeks, an intrusion of 3.6 mm was performed. The control group consisted of 15 patients who underwent no orthodontic treatment. Consequently, 58 incisors had no intrusion. The follow-up time between 2 measurements was 28 weeks. The findings clearly showed root shortening after intrusion. A mean resorption of 18% of the original root length was found. No correlation found between the amount of resorption and the amount and duration of intrusion. In combination with the apical deflection of the root, the nasal floor was occasionally a limiting factor for intrusion and this may have caused root resorption.

Levander E[51] (1988) did a study to investigate the risk of severe apical root resorption after orthodontic treatment with fixed appliances in relation to resorption after initial treatment, 6–9 months; and in relation to apical root form. The risk of severe apical root resorption in relation to resorption after 6–9 months of treatment was studied on 390 upper incisors in 98 consecutive patients (55 boys, 43 girls). Intra-oral radiographs before treatment, after 6–9 months and after treatment were evaluated. Root resorption after treatment was significantly related to the resorption after the initial 6–9 months. The results indicate a risk of severe resorption in teeth with minor resorption after 6–9 months. Even an irregular root contour after 6–9 months indicates a risk of severe resorption. The degree of root resorption in teeth with blunt or pipette shaped roots was significantly higher than in teeth with a normal root form.

McFadden W.M [55](1989) did a study of the relationship between incisor intrusion and root shortening. He evaluate the relationship between intrusion with low forces (25 gm) using utility arches in the bioprogressive technique and root shortening. Root shortening was found an average of

1.84 mm for maxillary incisors and 0.61 mm for mandibular incisors subjected to intrusive force. Furthermore, when extraction was a part of the orthodontic treatment, it was related to intrusion of maxillary incisors but not to intrusion of mandibular incisors. In the present study, it was found that intrusion with the utility arch type of technique is not related to amount of root shortening. The degree of root shortening was markedly higher in the maxilla than the mandible. In general, treatment time was the most significant factor for occurrence of root shortening. They concluded that control of treatment time is of importance especially when intrusion in the maxilla is performed.

Leif Linge[50] (1991) assessed the patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. Root lengths were measured in standardized intraoral radiographs from 485 consecutively treated patients, 11.5 to 25 years of age. Variables found to contribute significantly to apical root resorption were overjet, history of trauma to maxillary incisors before initiation of treatment, time of treatment with rectangular arch wires, time of treatment with Class II elastics, lip/tongue dysfunction, and/or history of finger-sucking habits persisting beyond the age of 7 yrs. Clinical application of an open activator was significantly correlated with overjet but negatively correlated with apical root resorption, with the use of rectangular arch wires and/or Class II elastics, and with total banding time.

Brudvik P [14] (1993) did a light microscopic investigation in order to study the initial phase of orthodontic root resorption in areas of pressure and, more specifically, to focus on the first cells that penetrate the root surface. Twenty-one upper first molars (rats) and 31 lower first molars (mice) were moved mesially by a fixed orthodontic appliance. Root resorption started in the circumference of the necrotic hyalinized tissue. In the central parts of the hyalinized zone frontal root resorption occurred 3-4 days later than in the periphery. The initial penetration of cells into precementum, cementum occurred at the peripheries or at a short distance from the peripheries of the hyalinized zone. These cells were TRAP-negative, indicating that they were not clasts or clast precursors. TRAP-positive cells were first observed in the bone marrow spaces. During the later stages mono- and multi-nucleated TRAP-positive cells were participating in active removal of the hyalinized tissue toward the root surface, and in resorption of cementum and dentin.

Levander Eva [52] **(1994)** did an investigation to evaluate the effect of a treatment pause on teeth in which apical root resorption was discovered after an initial treatment period of 6 months with fixed appliance. Forty patients with initial apical root resorption in 62 upper incisors were included in the study. In 20 patient’s treatment continued according to the original plan and in 20 patients active treatment was interrupted during a pause of 2–3 months. After the pause active treatment was resumed. Assessment of apical root resorption was performed on standardized radiographs taken with individual film holders. The amount of root resorption was significantly less in patients treated with a pause than in those treated without interruption.

Brudvik P [15](1994) did an investigation on the initial phase of root resorption associated with orthodontic overcompression of local areas of the periodontal ligament (PDL), indicated that a differentiation should be made between two stages: (1) the very first resorption occurring in the periphery of the main necrotic zone; and (2) the root resorption occurring on that part of the root surface situated beneath the main bulk of necrotic tissue. The study was done focusing on: (1) the possible association between the presence of necrotic tissue and root resorption; and (2) the cells that invaded and removed the necrotic tissue, as well as the cells that started to remove/resorb the cementum. The results indicate an association between the root resorption, and the presence and active removal of the hyalinized tissue. The majority of the cells involved in removal of the necrotic tissue and resorption of the root surface were multi-nucleated and TRAP-positive. It is hypothesized that multi-nucleated TRAP-positive cells (Tratrate acid resistant positive cells) when reaching the subjacent contaminated and damaged root surface after having removed necrotic tissue, continued to remove the cementum surface.

Owman-Moll P [64] (1995) studied the reparative potential of orthodontically induced root resorption. Sixty-four maxillary right and left first premolars in 32 patients (15 boys and 17 girls, mean age 13.7 years) were moved buccally with fixed orthodontic appliances and a continuous force of 50 cN (approximately 50 g), activated weekly for 6 weeks. The patients were divided into 4 groups of 8. Retention periods varied from 1 week to 8 weeks.

Histological preparations showed that root resorption affected all the test teeth. The percentage of resorptive areas that had begun to repair ranged from 28% after 1 week of retention to 75% after 8 weeks. The healing cementum was almost exclusively of the cellular type. Partial repair, with the resorption cavity walls only partially covered with cementum, was the most frequent type of repair during the first 4 weeks of retention (17% to 31%). Functional repair, with the total surface of the resorption cavity walls covered with varying thicknesses of cementum, dominated after 5, 6, 7 and 8 weeks of retention (33% to 40%). After 8 weeks, three out of four resorptive areas showed some degree of repair. Individual variations in healing potential were large.

Owman-Moll PY [65] (1996) did a clinical and histological study to investigate the effect on tooth movements and adverse tissue reactions (root resorption) when a fixed orthodontic appliance was activated with a controlled, continuous force of 50 cN («50 g) or with a four-fold larger force (200 cN«200 g). The first premolar on both sides of the maxilla in eight individuals, six boys and two girls (mean age 13.0 years), was moved buccally during 7 weeks with 50 cN and 200 cN alternately on the right or left side. During the first week a force reduction of 18 and 28 per cent (on average) was registered in the 50 cN and 200 cN group respectively. Root resorptions were registered in histological sections of the extracted test teeth with no significant difference in frequency or severity between the two forces used.

Akira Horiuchi [1] **(1998)** did a study to find out the correlation between cortical plate proximity and apical root resorption. He investigated the correlation between apical root resorption and the measured variables. His findings suggest that root approximation to the palatal cortical plate during orthodontic treatment could explain approximately 12% of the variance observed in the level of root resorption and the maxillary alveolar bone width about 2%. Tooth extrusion and crown lingualization also contributed to root resorption. They concluded that maxillary central incisor apical root resorption is influenced by root approximation to the palatal cortical plate during orthodontic treatment.

Taner [76]**(1999)** determined the amount of root resorption during orthodontic treatment, and examined the relationship between tooth movement and apical root resorption. Twenty-seven Class I and twenty seven Class II patients treated with edgewise mechanics following first premolar extractions were selected. The amount of apical root resorption of the maxillary central incisor was determined for each patient by subtracting the post-treatment tooth length from the pre-treatment tooth length measured directly on cephalograms. The results show that there was a mean of approximately 1 mm ($P<0.01$) of apical root shortening in Class I patients, but in Class II division I subjects the mean root resorption was more than 2 mm ($P<0.001$). The inter-group differences were statistically significant. No significant correlations were found between the amount of apical root resorption and tooth inclination, or the duration of active treatment.

Owman–Moll P [66] (2000) did an investigation to analyze factors that might be associated with orthodontically induced root resorption. After the buccal movement of maxillary premolars in 96 adolescents, the experimental teeth were extracted and subjected to histological analysis and measurement of resorbed root area. Fifty individuals, 18 boys and 32 girls, mean age 13.4 years, were selected and divided into two equal groups: the high-risk group based on measurements of the most severe root resorptions, and the low-risk group on measurements of mild or no root resorptions. After a preliminary screening of possible risk factors regarding root resorption, i.e. root morphology, gingivitis, allergy, nail-biting, medication, etc., only those subjects with allergy showed an increased risk of root resorption.

Glenn .T. Sameshima [32] (2001) did a study to determine which treatment factors are most clearly identified with external apical root resorption that is detectable on periapical radiographs at the end of orthodontic treatment. The records of 868 patients who completed fixed, edgewise treatment from experienced clinicians in private practice were examined. The horizontal and vertical displacement of the root apex of the maxillary central incisor was measured on cephalometric radiographs. Patients who underwent first premolar extraction therapy had more resorption than those patients who had no extractions or had only maxillary first premolars removed. Duration of treatment and the horizontal (but not vertical) displacement of the incisor apices were significantly associated with root resorption. No differences were found for slot size, archwire type, use of elastics, and types of expansion. They concluded that the clinician should exercise caution with those patients in whom extraction therapy is planned for overjet correction that requires above average treatment time. Finally, each clinician should be aware that the root resorption seen in one practice may be different from the root resorption found in another practice.

Weiland F [79] **(2003)** did a study to compare the effects of two frequently used archwires on tooth movement and root resorption. A total of 84 premolars in 27 individuals (10 boys, 17 girls, with a mean age of 12.5 years) were moved buccally with an experimental fixed orthodontic appliance. In a split mouth experimental design the premolar on one side was activated with a stainless steel wire with a buccal offset of 1 mm, which was reactivated every four weeks and the contralateral premolar was moved with a superelastic wire with a force plateau of 0.8–1 N. This wire had an initial activation of 4.5 mm and was not reactivated during the 12-week experimental period. At the end of the experimental period the teeth were extracted. The results show that the teeth activated with the superelastic wire moved significantly more than the teeth with the steel wire during the experimental period. The depth of the resorption lacunae did not differ significantly between the groups; however, perimeter, area, and volume of the resorption lacunae on the teeth of the 'superelastic group' were 140 per cent greater than on the teeth of the 'steel group'. It may be concluded that a greater amount of tooth movement occurred with superelastic wires, offering a force level of 0.8–1 N compared with stainless steel wires, with initially higher but rapidly declining forces in an experimental set up for a period of 12 weeks. The amount of root resorption was significantly larger in the superelastic group.

Daniel C S Ngan [22] (2004) did a retrospective twin study on the genetic contribution of orthodontic root resorption. The subjects consist of 16 monozygomatic twins and ten dizygotic twins. All twins had zygosity diagnosed using microsatellite analysis. Each twin pair had the same type of malocclusion and was treated with the same type of appliance and by the same clinician. The length of the maxillary incisors, mandibular incisors and mandibular molars were assessed both qualitatively and quantitatively on pre and post treatment panoramic films. Concordance estimates from the qualitatively and quantitatively measured external apical root resorption were 44.9 percent and 49.2 percent respectively for monozygomatic twins and 24.7 percent and 28.3 percent respectively for dizygomatic twins. Qualitatively and quantitatively determined estimates of concordance indicate a genetic component to root resorption.

Darendeliler MA [23] (2006) examined the physical properties of root cementum. and the extent of root resorption under areas of compression and tension, under light and heavy buccal tipping orthodontic forces. The sample consisted of 36 premolars in 16 patients. On 1 side, light (25 g) or heavy (225 g) buccal tipping orthodontic forces were activated for 28 days. The contralateral side in each patient served as the control (0 g). The teeth were extracted, disinfected, imaged under a scanning electron microscope, and analyzed with commercial stereo imaging computer software modified for this study. Buccal and lingual surfaces were divided into 3 equal regions: cervical, middle, and apical. The degree of resorption was correlated to the amount of surface area under compression or tension. He concluded that the buccal cervical region had 8.16-fold more root resorption in the heavy-force group compared with the light-force group ($P < .01$). Regions under compression had more root resorption than regions under tension. There was more resorption in regions under heavy compression than in regions under light compression ($P < .01$). There was also more root resorption in regions under heavy tension than in regions under light tension ($P < .01$).

Harris DA [34] (2006) , Aims in this prospective randomized clinical trial were to quantify, 3 dimensionally, the amount of root resorption when controlled light and heavy intrusive forces were applied to human premolars and to establish the sites where root resorption is more prevalent. Fifty-four maxillary first premolars, orthodontically indicated for extraction from 27 patients (left and right maxillary first premolars from each), were intruded for 28 days with buccal and palatal beta-titanium-molybdenum alloy 0.017 x 0.025-in cantilever springs. The patients were randomly divided into 3 groups, and various levels of force were used: group 1, heavy force (225 g) on 1 side and control force (0 g) on the contralateral side; group 2, light force (25 g) on 1 side and control force (0 g) on the contralateral side; group 3, light force (25 g) on 1 side and heavy force (225 g) on the contralateral side. The volume of the root resorption craters after intrusion was found to be directly proportional to the magnitude of the intrusive force applied. The results showed that the control group had fewer and smaller root resorption craters, the light force group had more and larger root resorption craters than the control group, and the heavy force group had the most and the largest root resorption craters of all groups. The mean volumes of the resorption craters in the light and heavy force groups were 2 and 4 times greater than in the control groups, respectively. The mesial and distal surfaces had the greatest resorption volume, with no statistically significant difference between the 2 surfaces.

Andreas Jagear [3] (2008) investigated the role of extra cellular matrix components, such as type I collagen, fibronectin, osteopontin (OPN) during cementum repair following experimentally induced tooth movement and to characterize the cells taking part in the regenerative process. The upper right first molars were moved mesially in 21 three month old male wistar rats using a coil spring with a force of 0.5 n. After 9 days the appliance was removed and the animals were killed in groups of three immediately after withdrawal of the force and 5, 7, 10, 12, 14, and 17 days later. Together with the disappearance of multi nucleated odontoclasts from the resorption lacunae signs of repair were visible 5 days after the release of the orthodontic force. The first signs of cementum repair were seen on day 10. The newly produced cementum was of the acellular extrinsic fiber type (AEFC) and reattachment was achieved with the principal periodontal ligament fibers oriented almost perpendicular to the root surface. The initial interface formed between the old and new cementum ,as well as the new AEFC, was characterized by strong immune reactions between the OPN and collagen I antibody, but only a weak immune reaction with the fibronectin antibody. Thus most of the cells associated with this reparative activity on the surface of the lacunae were differentiated PDL cells of the fibroblastic phenotype. Cells with a defined osteoblastic phenotype seemed to be of minor importance in this repair process.

Björn U. Winter [10] (2009) The objective was to examine these processes related to time and root development. Seventy-six premolars were divided into subgroups: 33 teeth were intruded and then extracted (G1); 25 teeth were intruded and then left in situ for varying periods before extraction (G2); 18 teeth served as the controls (G3). Resorption was observed over the whole root surface and increased with time. The occurrence increased to 100 per cent in both experimental groups after 36 days of intrusion. Resorptive lesions undergoing repair were seen in both groups, with significantly more repair in G2 (58 per cent) than in G1 (32 per cent). Active resorption and repair were sometimes seen at the same resorption site. Deposition of cellular and acellular cementum was found to the same extent over the whole root when repair took place. In some teeth, resorptive activity continued up to 10 days after removal of forces but on the other hand, repair of the resorbed area sometimes started during active movement. The individual variation in repair was much wider compared with resorption. The predentine layer in the apical area appeared not to be affected by the resorptive process.

Compositional changes of periodontium:-

Selvig K A[74] (1962) determined the age changes that takes place in the mineral content of cementum by chemical analysis in teeth with healthy periodontal tissue. Twenty teeth, among a large number of extracted teeth, were selected from each of the following groups: through twenty years, thirty-one to forty years, and over fifty years of age. Determination of the total Ca + Mg content can be used as an indication of the degree of mineralization of cementum. The phosphorus content was determined calorimetrically by a modification of the ammonium molybdate method. The Ca + Mg content, determined as calcium, ranged around a mean value of 26.2%, and the phosphorus content ranged around 12.2%. In the cervical cementum, the Ca + Mg content and the P content were higher in the oldest age group than in the two other age groups. If an increased mineral content per unit dry weight is observed, it is probably due to an increased degree of mineralization. Therefore, only in cases where increased mineral content is observed can valid conclusions be drawn with regard to changes in mineralization. Teeth from individuals more than fifty years of age, with no periodontal disease, showed an increased Ca + Mg and P content of the cementum in the cervical area of the roots. Since many samples from this group represented cementum that had been exposed to the oral cavity because of gingival recession, the hypothesis was offered that the saliva might cause a secondary mineralization to take place in exposed cementum. This investigation concluded that the age of the tooth should be taken into consideration when a comparison is made of the composition of teeth with diseased and healthy periodontal tissues.

Neiders M.E [61] (1972) studied 5 teeth to determine mineral content using an electron microprobe with scanning electron microscope attached to obtain data on the chemical composition of small selected region of tooth. And his quantitative analysis shows that calcium composition in the cervical is 25.6% and midroot region 26.0%. and composition of phosphorus is 12.9% in cervical and 13.5% in midroot region . And he concluded that calcium, phosphorus, and magnesium compositions obtained by electron microprobe analysis were within the range of values obtained by other analytic methods reported in the literature. The electron microprobe with scanning electron microscope attachment was used to obtain data on the chemical composition of small selected regions of the tooth that were typical of the morphology of that region.

Barton NS [7] (1987) A scanning electron microscope and electron probe study was carried out to compare root structure from deep within periodontal pockets with roots exhibiting no periodontal disease. To eliminate the possibility of extraneous ion introduction or deletion, no attempt was made to subject the roots to fixation, embedding, or dehydration prior to sectioning and viewing in the electron microscope. Mineral content and concentration were determined with an electron probe on two specimens per tooth. The following conclusions can be drawn from the study: (1) Consistent and repeatable qualitative electron probe analyses can be performed on human teeth with minimal specimen preparation. (2) Minerals consistently found were P, Ca, Cu, Zn, Mg and Na. They were in similar concentrations throughout the area analyzed. (3) Mg and Cu showed higher values in the nondiseased teeth. (4) There were no differences in concentrations for Ca, P, Zn, and Na between roots exposed to a periodontal pocket and nondiseased roots.

Attila G [6] (1996), determined the presence of certain inorganic elements in various cementum surfaces. The electron probe analysis appears to be the most suitable technique for examining elemental composition within localized small areas of mineralized tissue, it was used to determine the mineral contents of nondiseased, periodontally diseased and root planning applied diseased cementum surfaces were determined by electron probe. Calcium (Ca) and phosphorus (P) were the elements searched for. Surface morphological features of these cementum surfaces were also examined. Electron probe analysis indicated an elevated Ca and P content in diseased cementum surfaces. Root planning of these surfaces did not reduce the mineral contents of these root surfaces. Our findings indicate that on the cementum surfaces affected by periodontal disease inorganic contents were altered and the hypermineralized layer in diseased cementum may play a role in preventing total diseased cementum removal by root planning.

Khounganian R.M [42] (2006) evaluated the differences between healthy and periodontally diseased root cementum including surface characterisitcs , presence of endotoxins and the distribution of various elements within healthy and diseased cementum using light and scanning electron microscopy and energy dispersive x ray analysis . The minerals most often detected in the specimens were phosphorus (P), calcium (Ca), copper (Cu), zinc (Zn), magnesium (Mg), and occasionally sodium (Na). Calcium (30.05%) and phosphorus (14.70%) contents of the diseased cementum surface were higher in comparison to non-diseased cementum of Ca (29.69%) and P (13.72%) . He concluded that the exposure of the root surface to the oral environment by recession and pocket formation results minimal alteration in root surface mineral content, and those pathological root surface alterations associated with periodontitis are due to contamination by bacterial plaque.

Kodaka T [45] (2002) examined afibrillar cementum (AFC) and cementicle-like structures (CLS) in human teeth by scanning electron microscopy and energy-dispersive X-ray microanalysis, and studied the fine structure, distribution pattern and calcification of AFC in human teeth and also those of cementicles (CEC) in order to make a comparison with AFC. In his present SEM-EDX data their calcification values were lower than that of the enamel and higher than those of the fibrillar acellular and cellular cementum. It has been reported that as the concentration of collagen fibrils becomes lower, the degree of calcification will become higher in collagenous calcified matrix, Calcium in fibrillar cementum 25.6% and afibrillar cementum 27.1%, and Phosphorus is 12.2% in fibrillar and 13.7% in AFC, and Ca/P ratio of 1.64% in fibrillar and 1.53% in AFC. Therefore, the organic matrixes like Ca and P content of AFC, CLS and CEC containing no collagen fibrils will be lower amounts than that of the fibrillar cementum. In the enamel fissures, the calcification values of AFC and CLS were higher than that of AFC in the root furcations and similar to that of the CEC. This means that secondary calcification, probably derived from blood, tissue fluid or saliva, might occur less in the AFC of root furcations than in the others.

Rex Teriko et al [72] (2005) performed a quantitative analysis of the calcium (Ca), phosphorus (P), and fluoride (F) concentrations in human first premolars. In 18 maxillary or mandibular first premolars mean age 13.9 years (range, 11.7-16.1 years). After extraction, the teeth were prepared for electron probe microanalysis. The Ca, P, and F concentrations were measured on the buccal and lingual surfaces at the midpoint of the cervical, middle, and apical thirds of the root from the outer to middle to inner third of the cementum, and he concluded that In first premolar cementum, there was significant interindividual variation in the Ca, P, and F concentrations. There was no significant difference in the Ca, P, and F concentrations of cementum between the buccal and lingual surfaces, except for a significantly higher F content at the cervical region on the buccal surface. There was a decreasing gradient in the Ca, P, and F concentrations from the cervical to the apical third of the root, which was highly significant from the cervical to middle third and from the middle to apical third, except for F, for which there was no significant difference from the cervical to the middle third on the lingual surface. There was a significant increasing gradient in the Ca and P concentrations from the outer to inner third of cementum at the cervical and middle thirds of the root but no significant difference at the apical third of the root. For F, there was a significant decreasing gradient from the outer to the inner third of cementum at the cervical, middle and apical thirds of the root.

Gonçalves PF [33] **(2005)** reviewed developmental and structural characteristics of cementum, a unique avascular mineralized tissue covering the root surface that forms the interface between root dentin and periodontal ligament. Acellular afibrillar cementum covers minor areas of the enamel, particularly at and along the cementoenamel junction. The areas and location of acellular afibrillar cementum vary from tooth to tooth and along the cementoenamel junction of the same tooth. Cellular intrinsic fiber cementum is found in the furcation, on the apical root portion, in old resorption lacunae and in root fracture sites, only cellular intrinsic fiber cementum can repair a resorptive defect of the root in a reasonable time, due to its capacity to grow much faster than any other type of cementum. Acellular extrinsic fiber cementum is mainly found on cervical and middle root portions, covering 40% to 70% of the root surface. It serves the exclusive function of anchoring the root to periodontal ligament. Acellular extrinsic fiber cementum appears more highly mineralized than cellular intrinsic fiber cementum and cellular mixed stratified cementum. For cementum regeneration periodontal ligament may be one source of cementoblasts progenitor cells in adult humans. Cementoblasts may also be derived from stem cells present in the periodontal ligament, gingiva, and alveolar bone.

Ranjith S [70] (2005) determined the morphology and mineralization of Sharpey's fibers in bundle bone of humans with reference to interdental area observed under scanning electron microscope (SEM) and the constituent elements were analyzed using energy dispersive X- ray spectroscopy (EDS). Human interdental septum between mandibular 1st and 2nd molar teeth of either side was chosen for the study. A total of five samples were taken front male patients of age group of 40 to 50 years. The normal interdental bone was obtained from edges of postoperative biopsy specimen of patients who underwent hemi - mandibulectomy for various reasons. The observation of samples was done at two different levels, crestal and apical. The values of weight % and in the apical part of interdental septum for, phosphorus 22.52 and, and calcium 53.72 and were found to be lesser than the crestal part for 31.00 for phosphorus, and 57.88 for calcium respectively. The mean difference was found to be statistically significant and supports the SEM and EDXA finding that the crestal region is more calcified or mineralized than the apical region of the interdental alveolar bone.

Materials and Methods

MATERIALS AND METHODS

10 patients (5 males, 5 females, mean age of 16.2years; range 13.5 - 21.6 years) who reported to the department of orthodontics at Ragas dental college, were screened for the study. Patients with Severe tooth size arch length discrepancy requiring 1st bicuspid extraction for orthodontic treatment with a fixed appliance were considered for the study. The study protocol was approved by the institutional research ethics committee.

INCLUSION CRITERIA

1. Children/adolescent in permanent dentition of either gender
2. No caries in 1st bicuspid
3. No periodontal pathology in the region of 1st bicuspid
4. Root formation completed in 1st bicuspid.

EXCLUSION CRITERIA

1. Pervious orthodontic history
2. Patients who have undergone root canal therapy
3. Dilacerated teeth
4. Patients under medication for systemic disorders and pregnancy
5. Patients under steroid therapy

All the patients fulfilled the inclusion and exclusion criteria in this prospective study.

MATERIALS

1. Pre-operative models
2. 0.021” X 0.025” Stainless Steel & 0.017’’X 0.025’’ Elgiloy wire for stent preparation
3. Pre-operative, after wire guide stabilization and post-operative intraoral peri-apical radiographs (Parallel cone technique, Kodak E- speed film)
4. Miniscrew(1.6mm diameter, 7mm length, AbsoAnchor microimplant, Dentos, Korea)
5. Pilot drill bur (1.2mm, Dentos, Korea)
6. Standard long handle driver
7. Micro motor hand piece
8. Saline
9. Local anesthetic solution (Lidocaine 2% A, 1:100,000)
10. Syringe and needle
11. Extraction forceps
12. Formalin 4%
13. Ethanol
14. Disk bur to split the tooth
15. Stereomicroscope (Laborlux B)
16. Scanning Electron microscope with EDAX detector system (Hitachi)

METHODS

PRE SURGICAL PROCEDURE

FABRICATION OF THE WIRE GUIDE FOR PLACEMENT OF MINISCREWS

Patient's pre-operative models and intra-oral peri-apical radiographs (Parallel cone technique) was taken to estimate the length of the 1^{st} bicuspid for the fabrication of custom made wire guide. The wire guide comprised of a stem 0.021" X 0.025" Stainless Steel strip to one end of which a horizontal arm was welded. The horizontal arm carries series of vertical guides made of 0.017''X 0.025'' Elgiloy which was welded at an interval of 1mm to each other. The wire guide permitted seating of the stem into the 1^{st} bicuspid bracket to stabilize it, and would allow the horizontal arm to rest in the gingival sulcus. A peri-apical radiograph (Parallel cone technique) was taken again after the wire guide was stabilized in the 1^{st} bicuspid bracket and helped in the appropriate placement of miniscrew. The image of the wire guide on the peri-apical radiograph for root contact was verified clinically with the vertical guides facilitating the assessment of mesiodistal positioning of miniscrew.

SURGICAL PROCEDURE

The miniscrews used for intentional root contact were of self-drilling design, 1.6mm in diameter and 7mm in length (Dentos, Korea) for each patient, and the miniscrew was inserted on the distal side of the buccal root surface of the 1st bicuspid. The miniscrew was inserted under local anesthetic (2% lidocaine with 1:100,000 epinephrine) on the same day based on the radiographic assessment. A punch was made on the attached gingiva with a periodontal probe on the predetermined site based on the radiographic assessment. With the wire guide in place a hole of 0.9mm diameter was drilled using No.2 round bur to make indentation through the cortex with a slow speed hand piece under continuous saline irrigation. Care was taken to ensure that there was only single contact. The miniscrews were placed using a manual torque tester. The resistance felt during miniscrew placement indicated that a contact with the root has occurred and this was further confirmed with perapical radiograph. After root contact was established the miniscrew was immediately removed.

Tooth extracted immediately after root damage with miniscrew were considered as a control group. The repair of periodontal apparatus and the root structure that was damaged were allowed to take place for varying length of time. Based on the healing period teeth were classified as Group A (4 weeks), Group B (8 weeks), Group C (12 weeks). After reparative healing period the experimental 1st bicuspids were extracted.

Immediately after extraction, the damaged surfaces were identified on distal surface of 1^{st} bicuspid using a stereomicroscope. The 1^{st} bicuspid was placed in 4% neutral buffered formalin and stored for 24 hours. The samples were dehydrated by ascending grades of ethyl alcohol starting from 70% to 100% of absolute alcohol and before 10 minutes of SEM examination the samples were allowed to dry completely.

The samples were then mounted on aluminum stub, sputter-coated with Gold target, and examined under SEM (HITACHI, TMP/RP BASED VACUUM SYSTEM) operated at 0.3 to 30 kV. And compositional elements were quantitatively analysed by using EDAX detector system (LN2 Free, Peltier Cooled 139eV)

STATISTICAL ANALYSIS

Patient demographics of age, gender, healing period in weeks and the mean repair were entered and analysed using SPSS version 16.0 (SPSS-IBM Inc., Chicago, IL, USA). Descriptive statistics including means, standard deviations, and ranges. Paired-student t test was used to evaluate the mean composition of elements in repair and undamaged area in Group A (4 weeks), Group B (8 weeks), and Group C (12 weeks). One way ANOVA was employed for the elemental ratio between repair and undamaged area for 3 groups and for calcium phosphorus ratio in repair and undamaged area during weeks. Post-HOC Bonferoni test was used for comparision of elements between the groups in repair area and for undamaged area. At this level to allow for multiple comparisons, statistical significance was at the $P < 0.05$ level.

Photographs

ARMAMENTARIUM

Figure 1 – 19 × 25 S.S &TMA wire guide, TAD’s, Pilot drill bur, Standard long handle driver, Micromotor hand piece, Saline, Local anesthetic solution, Artery forceps, disc bur

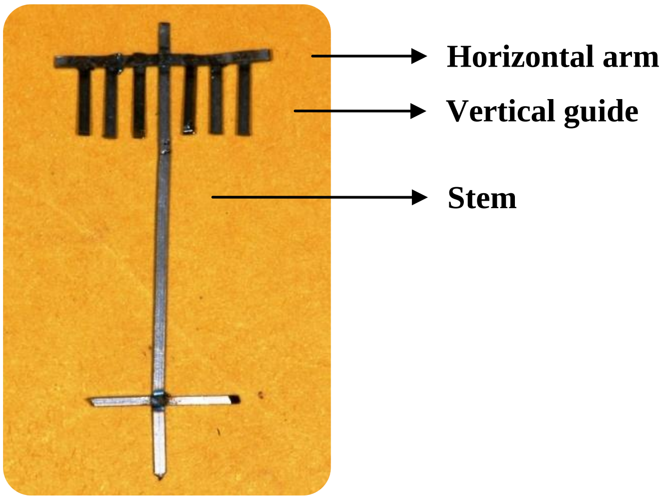


Figure 2 – Wire guide for miniscrew placement

Figure 3a – Intraoral periapical radiograph(Parallel cone technique) with the wire guide

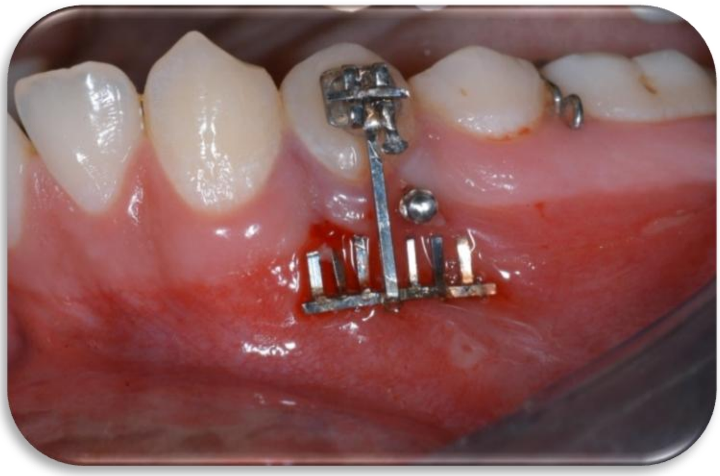


Figure 3b – Miniscrew placed on the distal root surface of lower left first bicuspid

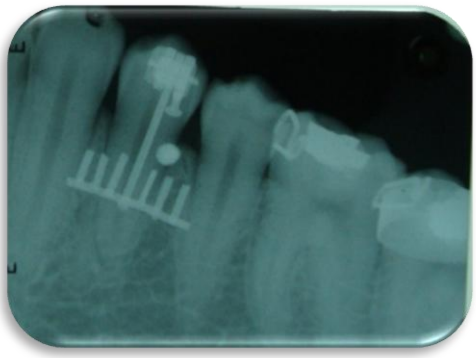


Figure 3c- Radiograph after miniscrew contacting

Figure 4 – Confirmation of root contacted with TAD using a stereomicroscope

Figure 5a- 4% neutral buffered formalin for tissue fixing

Figure 5b- Ethanol for dehydration

Figure 6a-Ion sputter for Gold coating

Figure 6b- Samples after coated with Gold ion on mounting aluminum stub

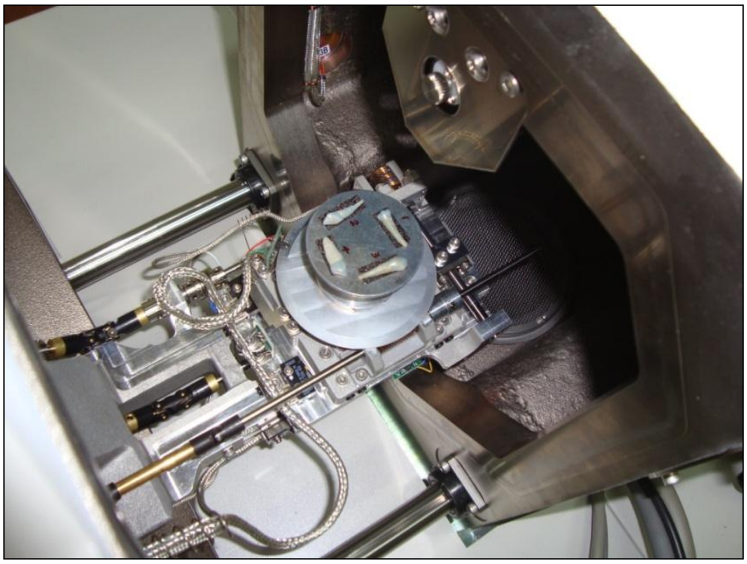


Figure 7a- Samples loading in scanning electron microscope

Figure 7b- Samples analysed by Scanning electron microscope and Energy dispersive X-ray analyser

Results

RESULTS

10 cases (5 males, 5 females) with a mean age of 16.2 years (13.5 -21.6 years) were included in the study. In each subjects one Ist bicuspid served as control (n=10) and remaining three Ist bicuspids served as experimental samples (n=30). The cases fulfilled the inclusion and exclusion criteria in this prospective study. None of the subjects complained of pain after intentional root contact with miniscrew. Extractions of teeth were carried out in 4th, 8th and 12th week as planned. Evidence of root damage with miniscrews was assessed using the stereomicroscope in all extracted teeth.

I Scanning electron microscopic evaluation (SEM);

Tooth extracted immediately after root damage with miniscrew were considered as control sample, which was latter compared with the experimental group of the same patient for qualitative analysis.

Control group (Immediately after damage)

At 5X magnification root damage is seen and at 25X magnification it is clearly visible. There was clear demarcation between the damaged area and undamaged area at 1000X magnification. At higher magnification of 2000X there was denuded dentin, cementum surface and devoid of periodontal ligament in damaged area; and intact periodontal ligament in adjacent undamaged area was evident.

Group A (4th week)

At 5X and 35X magnification damaged root surface is seen, during 4th weeks of repair period after damage, stress cracks were evident adjacent to damaged area at 60X magnifications, a shallow resorption crater was evident. And at higher level of 500X and1000X magnifications, there was no apparent denuded dentin and cementum surfaces were observed which was covered by calcified matrix. The immature organic fibers are seen, which shows an early sign of repair were visible in the resorption lacunae and regeneration of periodontal ligament and cementum will takes place.

Group B (8th week)

During 8th week of repair period , damaged root surface by miniscrew is clearly visible at 35X magnification, at 60X magnification the stress cracks were still evident adjacent to damaged area. At higher magnification of 150X more of new fibers were observed at the bottom of the resorption crater, and more immature organic fibers become matured at subsequent higher magnifications of 500X and 2000X were evident when compared to 4th week.

Group C (12th week)

During 12th week of repair period, the damaged area is evident at 5X and 25X magnification and stress cracks were not evident at border of defect even up to the magnification of 500X. And at higher magnification of 1000X, the damaged area of collagen fibers had reorganized completely and almost fully covered the damaged area. These fibers were apparently fully matured and had functionally repaired the defects when compared to 4^{th} and 8^{th} week groups. Morphological variation between damaged and undamaged area hardly noticeable, and normal collagen structure of the periodontium was observed in all resorption craters.

II <u>Energy Dispersive X-ray Analysis (EDX);</u>

No control group was taken separately for EDX analysis, the elemental changes occurring during the repair process were analysed and it was compared with the undamaged area of the same tooth around 1mm adjacent to the damaged area. The X –ray intensity counts obtained at each point were converted to percentage with standard magnification of 100X. The elements detected in the specimens were calcium (Ca), Phosphorus (P), Sodium (Na), chloride (cl), carbon(c), oxygen (O).

Table 1: Quantitative analysis of elements in repair area and undamaged area;

The mean value of Calcium in repaired area and adjacent undamaged area in 4th week group was 29.38± 1.37 and 26.88± 1.67, 8th week group was 30.79±5.48 and 28.92±3.43 and 12th week group was 28.86± 1.44 and 28.33± 1.3 respectively.

Phosphorus in repaired area and adjacent undamaged area on 4th week group was 14.73±1.63and 13.33±1.76, 8th week group 14.22±1.10 and 13.76±1.17, in 12th week group 14.09±1.36 and 13.59±1.67 respectively.

There was increased concentration of organic and all inorganic elements in repaired area when compared to adjacent undamaged area but there was no significant difference between 4th, 8th and 12th week in repaired and undamaged area.

Table 2: Elemental ratio between repair and undamaged area;

There was periodically decreasing gradient of calcium concentration between repaired area and adjacent undamaged area during 4th,8th and 12th week groups, exhibited a ratio of 1.09±0.04,1.06±0.06 and 1.02±0.01 respectively, which was statistically significant (P=0.001).

There was periodically decreasing gradient of Phosphorus concentration between repair area and undamaged area during 4th,8th and 12th week groups, exhibited a ratio of 1.11±0.10, 1.09±0.09 and 1.04±0.06 respectively, but it was not statistically significant (P=0.193).

There was periodically increasing gradient of Sodium concentration between repair area and undamaged area from 4th to 12th week group, exhibited a ratio of 1.20± 0.32, 1.29± 0.81 and 1.41± 0.64 respectively, but it was not statistically significant (P=0.746).

Chloride, Carbon and Oxygen between repair area and undamaged area from 4th to 12th week group, didn't show any periodically increasing or decreasing gradient of concentration in all experimental weeks.

Table 3: Calcium and phosphorus ratio in repair and undamaged area;

Results showed that Ca/P ratio during 4th, 8th and 12th week were not statistically significant in repaired area (P=0.541) and also in adjacent undamaged area (P=0.545).

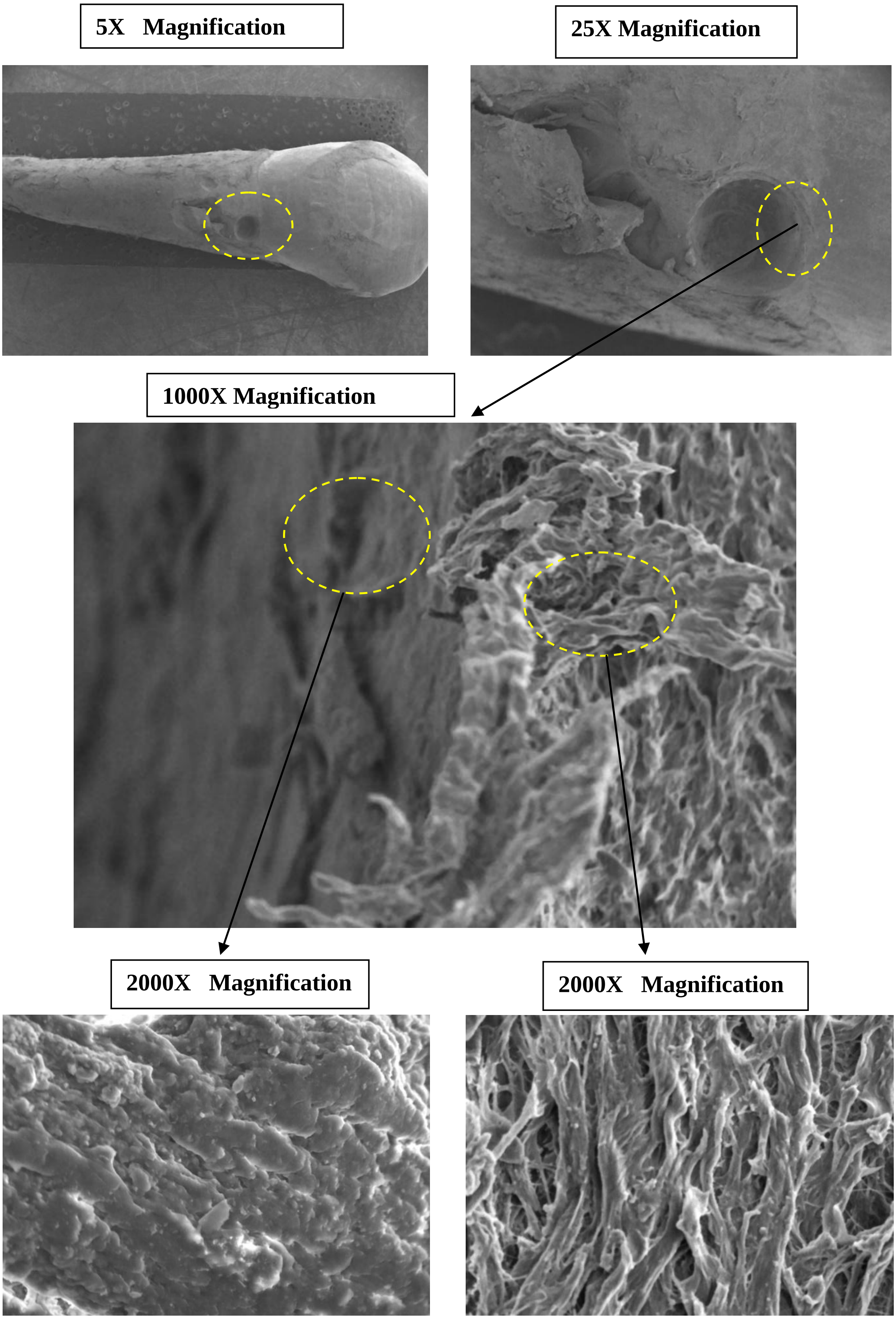
Table 4: Comparison of elements between the groups during repair period:

Results showed that there was no statistically significant difference in calcium, phosphorus, sodium, chloride, carbon, oxygen, between 4^{th}, 8^{th} and 12^{th} week groups during repair period.

Table 5: Comparison of elements between the groups in undamaged area:

Results showed that there was no statistically significant difference in calcium, phosphorus, sodium, chloride, carbon, oxygen, between 4^{th}, 8^{th} and 12^{th} week groups in undamaged area.

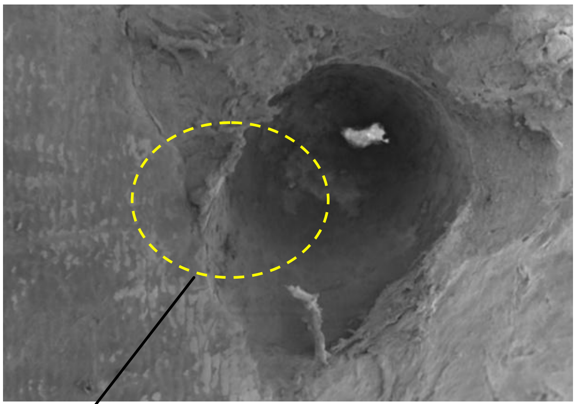
Control (Immediately after damage)



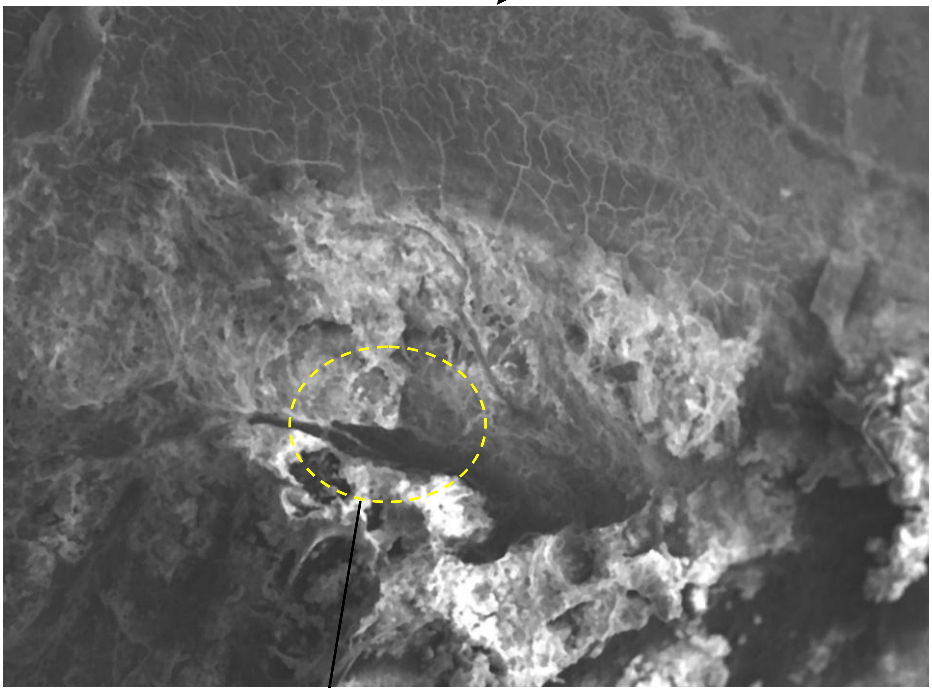
Group-A (4th week)

5X Magnification

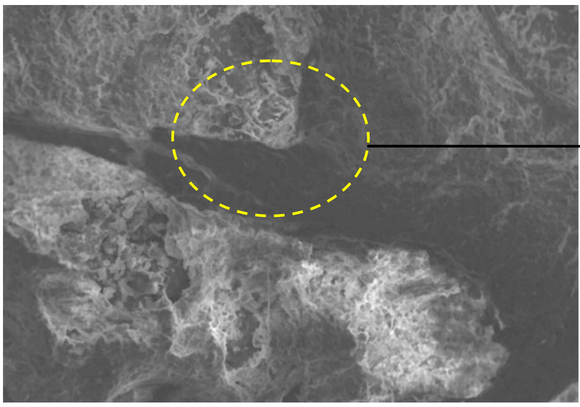
35X Magnification



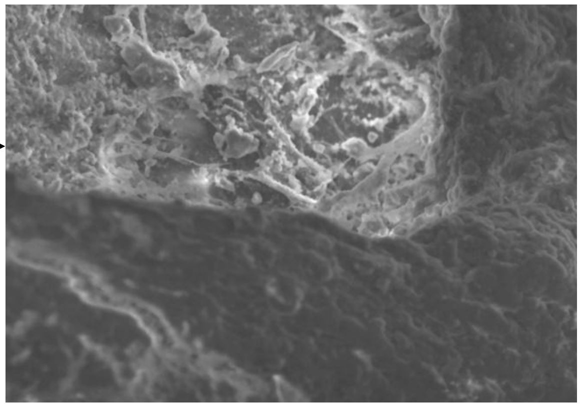
60X Magnification



500X Magnification

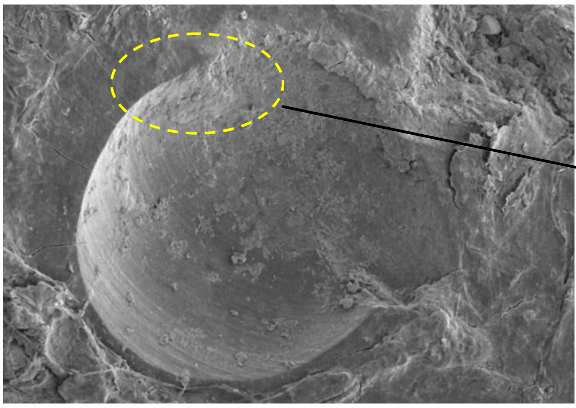


1000X Magnification

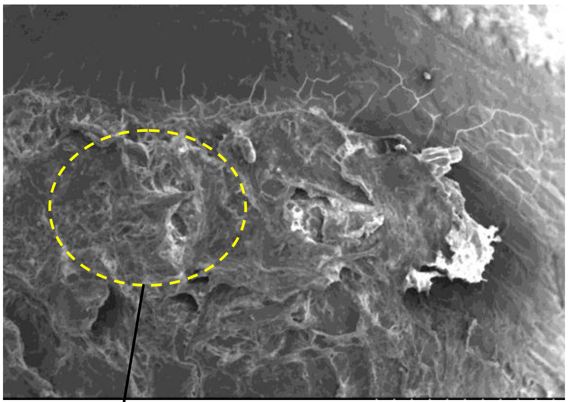


Group-B (8th week)

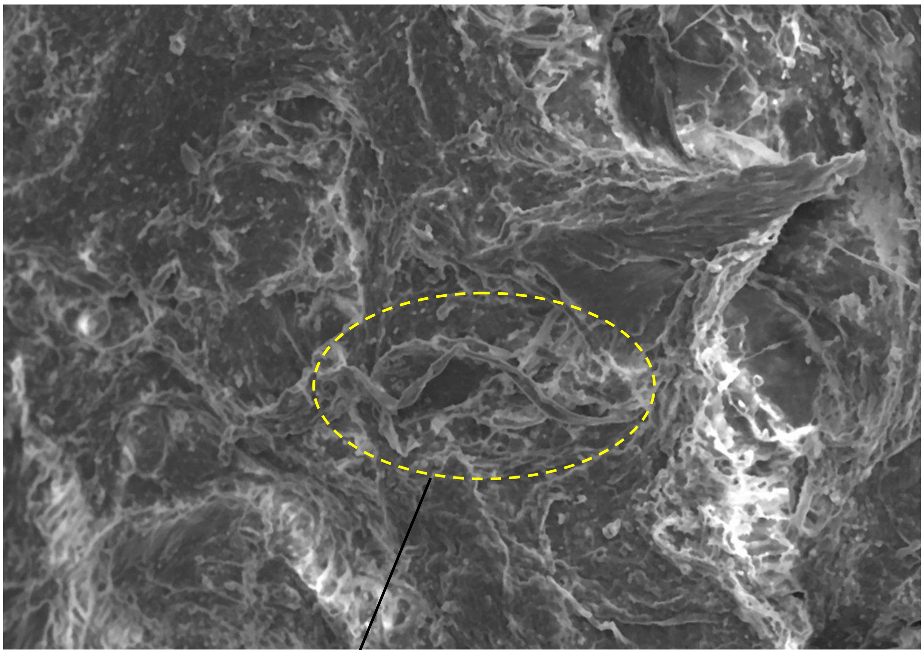
35X Magnification



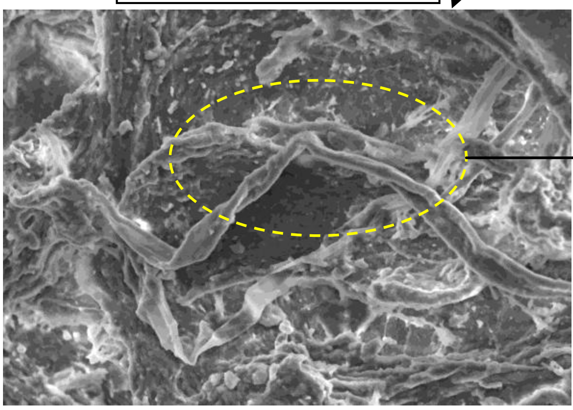
60X Magnification



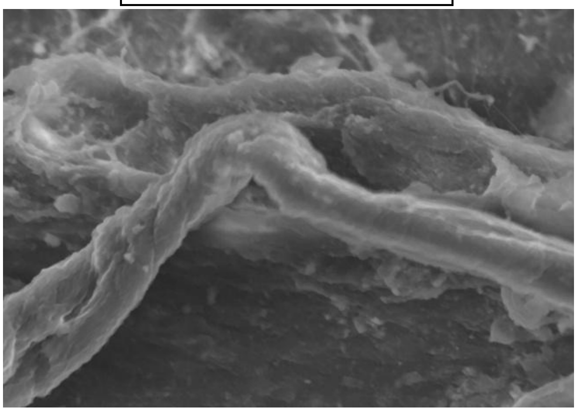
150X Magnification



500X Magnification

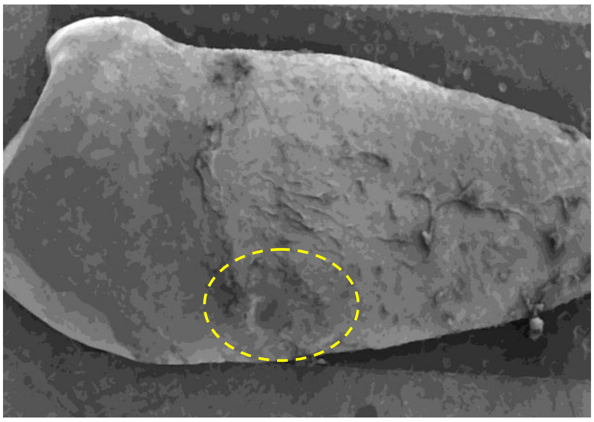


2000X Magnification

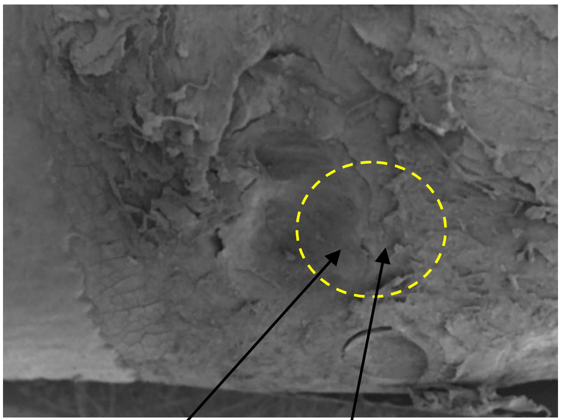


Group-C (12th week)

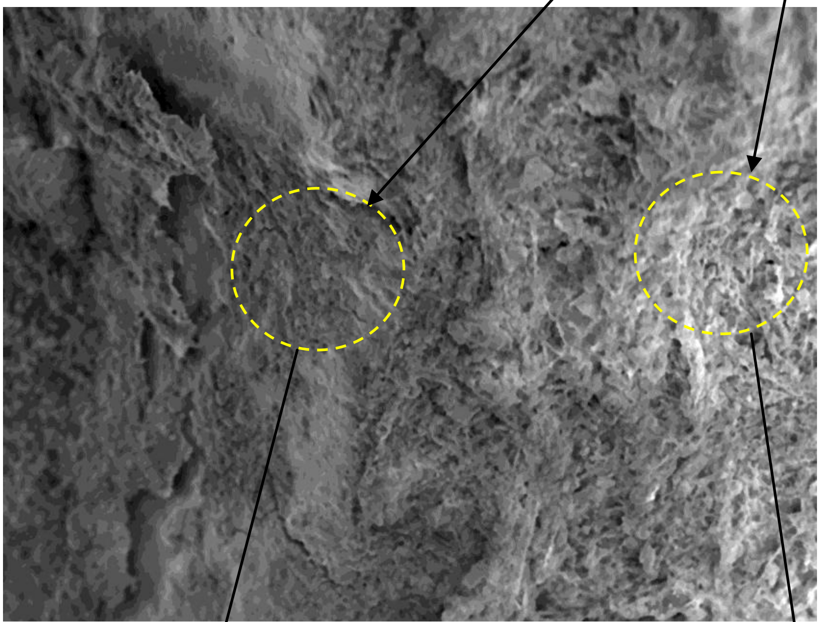
5X Magnification



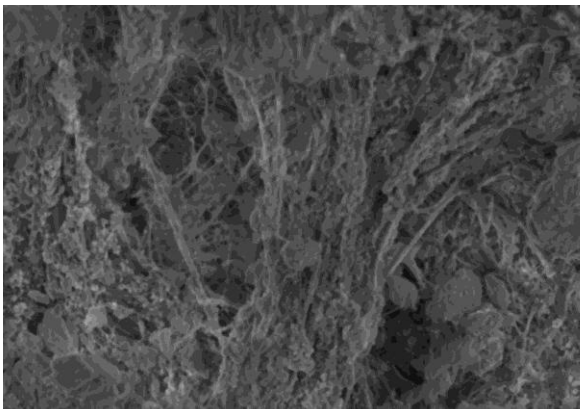
25X Magnification



500X Magnification



1000X Magnification



1000X Magnification

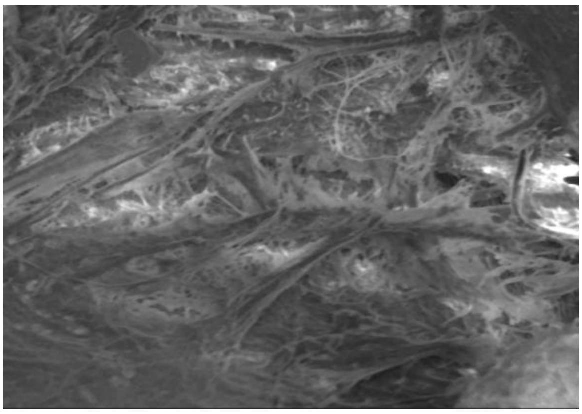


TABLE 1: Mean composition of elements in repair and undamaged area in weeks

		4 weeks		8 weeks		12 weeks		P value
		Mean	Std	Mean	Std	Mean	Std	
Calcium	Repair	29.38	1.37	30.79	5.48	28.86	1.44	0.426
	Undamaged	26.88	1.67	28.92	3.43	28.33	1.39	0.154
Phosphorus	Repair	14.73	1.63	14.22	1.10	14.09	1.36	0.552
	Undamaged	13.33	1.76	13.16	1.17	13.59	1.67	0.826
Sodium	Repair	0.96	0.37	0.76	0.31	0.93	0.38	0.414
	Undamaged	0.80	0.16	0.69	0.25	0.72	0.22	0.47
Chloride	Repair	2.88	0.69	2.57	0.77	3.02	0.59	0.339
	Undamaged	2.45	0.57	3.05	2.11	3.27	1.48	0.469
Carbon	Repair	24.78	3.11	20.37	9.44	26.13	3.53	0.106
	Undamaged	24.71	3.01	21.02	8.55	27.65	10.52	0.199
Oxygen	Repair	25.56	2.83	25.30	2.51	25.81	1.72	0.893
	Undamaged	24.78	1.82	24.27	2.76	25.02	1.42	0.716

TABLE 2: <u>Elemental ratio between repair and undamaged area.</u>

		Mean	Std. Deviation	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
Calcium	Week 4	1.09	0.04	1.07	1.12	**0.001 ***
	Week 8	1.06	0.06	1.02	1.10	
	Week 12	1.02	0.01	1.01	1.03	
Phosphorus	Week 4	1.11	0.10	1.04	1.18	0.193
	Week 8	1.09	0.09	1.02	1.15	
	Week 12	1.04	0.06	1.00	1.08	
Sodium	Week 4	1.20	0.32	0.97	1.43	0.746
	Week 8	1.29	0.81	0.71	1.87	
	Week 12	1.41	0.64	0.96	1.87	
Chloride	Week 4	1.23	0.39	0.95	1.51	0.283
	Week 8	1.00	0.38	0.73	1.27	
	Week 12	1.01	0.28	0.82	1.21	
Carbon	Week 4	1.00	0.07	0.96	1.05	0.984
	Week 8	1.03	0.42	0.72	1.33	
	Week 12	1.02	0.23	0.86	1.18	
Oxygen	Week 4	1.03	0.08	0.98	1.09	0.843
	Week 8	1.05	0.09	0.98	1.11	
	Week 12	1.03	0.02	1.01	1.05	

***P value ≤ 0.05 indicating statistical significance.**

TABLE 3 Calcium phosphorus ratio in repair and undamaged area during weeks

		Mean	Std. Deviation	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
Ca/P Repair	Week 4	2.01	0.22	1.86	2.17	0.541
	Week 8	2.18	0.49	1.84	2.53	
	Week 12	2.07	0.27	1.88	2.26	
Ca/P Undamaged	Week 4	2.04	0.24	1.87	2.21	0.545
	Week 8	2.23	0.50	1.87	2.59	
	Week 12	2.12	0.37	1.86	2.39	

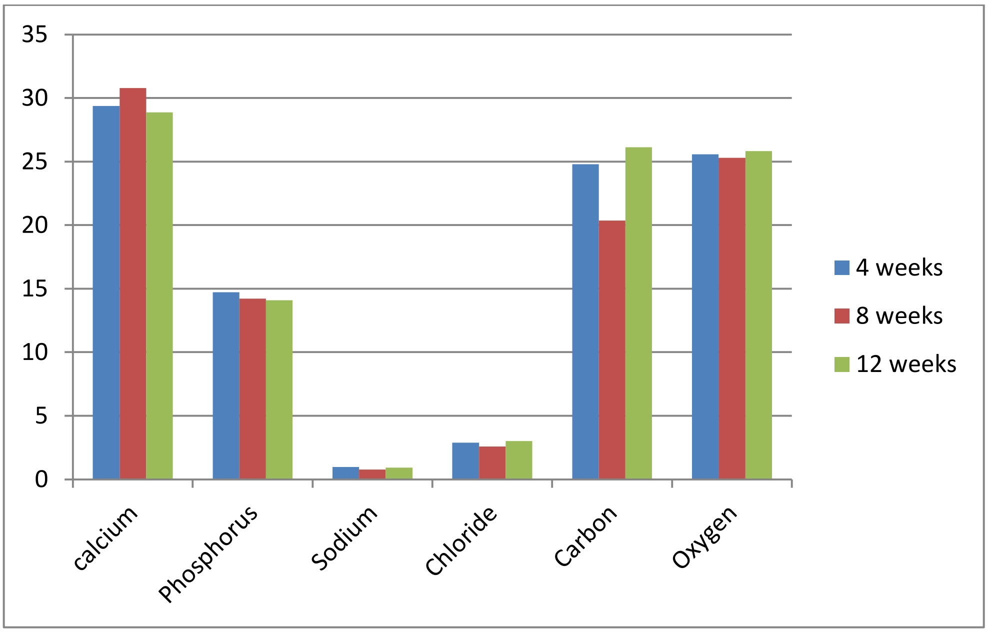
TABLE 4: Comparison of elements between the groups in repair area:

Dependent Variable	(I) Week	(J) Week	Mean Difference (I-J)	P value
C-P Repair	Week 8	Week 4	0.17	0.85
	Week 12	Week 4	0.05	1.00
	Week 12	Week 8	-0.12	1.00
Calcium Repair	Week 8	Week 4	1.41	1.00
	Week 12	Week 4	-0.52	1.00
	Week 12	Week 8	-1.93	0.63
Phosphorus Repair	Week 8	Week 4	-0.51	1.00
	Week 12	Week 4	-0.64	0.92
	Week 12	Week 8	-0.13	1.00
Sodium Repair	Week 8	Week 4	-0.20	0.67
	Week 12	Week 4	-0.03	1.00
	Week 12	Week 8	0.17	0.89
Chloride Repair	Week 8	Week 4	-0.31	0.96
	Week 12	Week 4	0.14	1.00
	Week 12	Week 8	0.45	0.46
Carbon Repair	Week 8	Week 4	-4.41	0.35
	Week 12	Week 4	1.35	1.00
	Week 12	Week 8	5.76	0.13
Oxygen Repair	Week 8	Week 4	-0.26	1.00
	Week 12	Week 4	0.26	1.00
	Week 12	Week 8	0.51	1.00

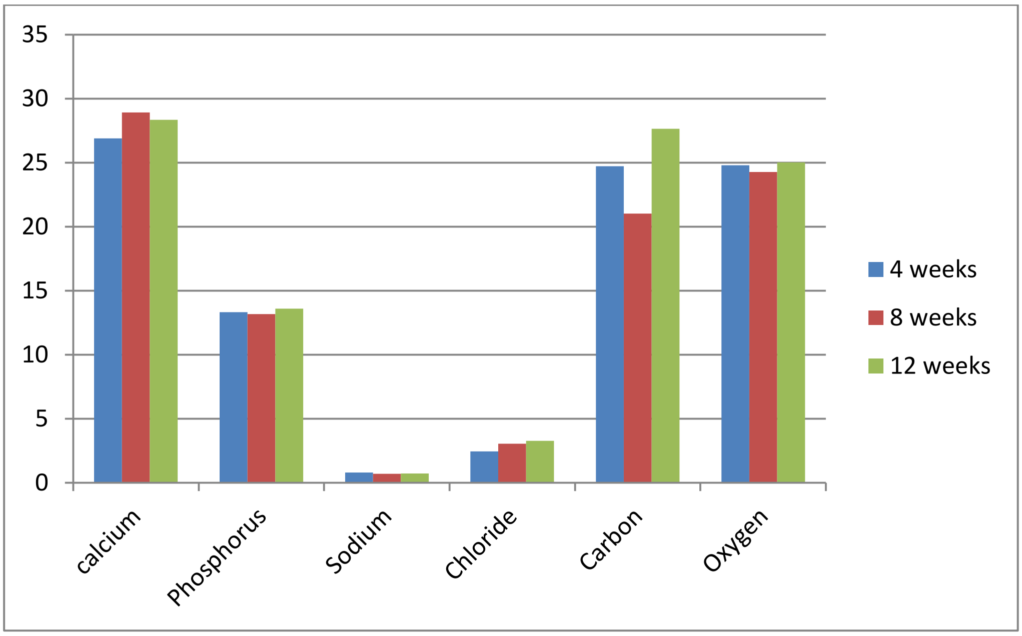
TABLE 5 Comparison of elements between the groups in undamaged area

Dependent Variable	(I) Week	(J) Week	Mean Difference (I-J)	P value
C-P Undamaged	Week 8	Week 4	0.19	0.83
	Week 12	Week 4	0.08	1.00
	Week 12	Week 8	-0.11	1.00
Calcium Undamaged	Week 8	Week 4	2.04	0.19
	Week 12	Week 4	1.46	0.53
	Week 12	Week 8	-0.59	1.00
Phosphorus Undamaged	Week 8	Week 4	-0.17	1.00
	Week 12	Week 4	0.26	1.00
	Week 12	Week 8	0.43	1.00
Sodium Undamaged	Week 8	Week 4	-0.11	0.73
	Week 12	Week 4	-0.09	1.00
	Week 12	Week 8	0.03	1.00
Chloride Undamaged	Week 8	Week 4	0.60	1.00
	Week 12	Week 4	0.82	0.71
	Week 12	Week 8	0.22	1.00
Carbon Undamaged	Week 8	Week 4	-3.69	0.94
	Week 12	Week 4	2.94	1.00
	Week 12	Week 8	6.63	0.23
Oxygen Undamaged	Week 8	Week 4	-0.51	1.00
	Week 12	Week 4	0.24	1.00
	Week 12	Week 8	0.75	1.00

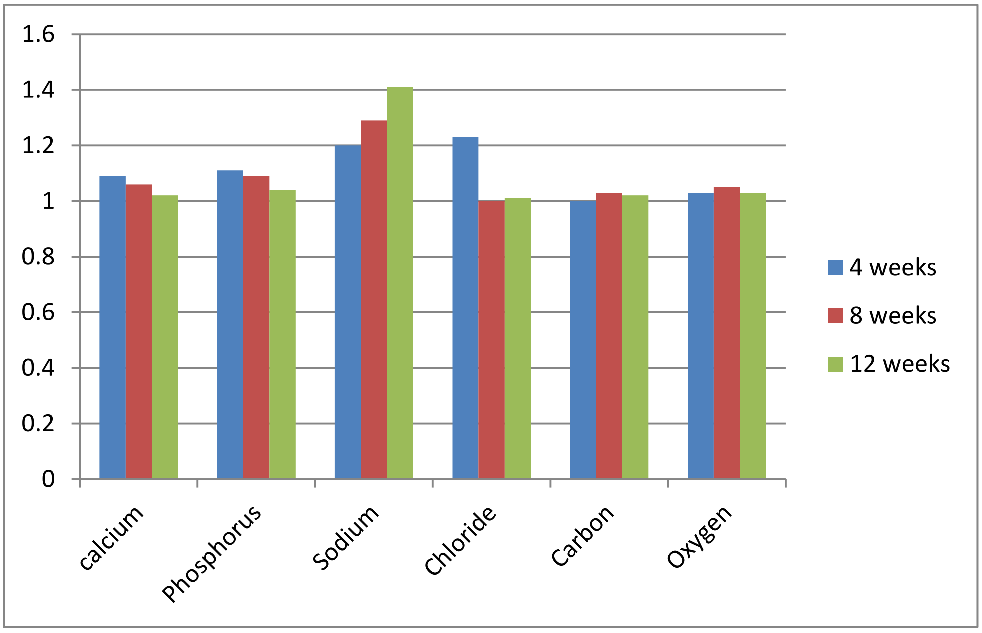
Graph 1 A – Mean composition of elements in repair area during 4, 8 and 12 weeks



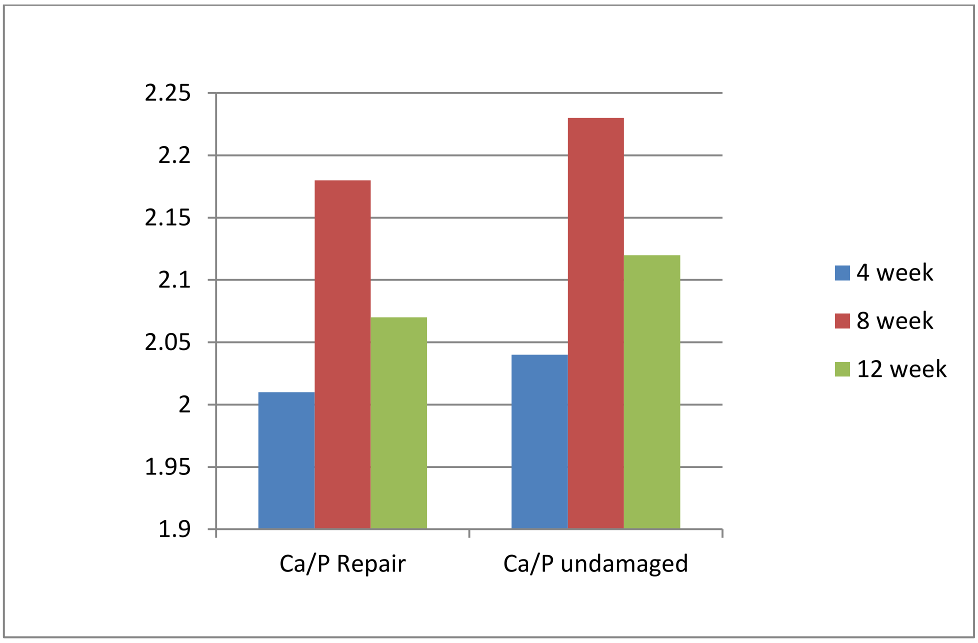
Graph 1 B-- Mean composition of elements in undamaged area during 4, 8 and 12 weeks



Graph 2- Elemental ratio between repair and undamaged area in 4,8 and 12 weeks



Graph 3- Calcium : Phosphorus ratio in repair and undamaged area during 4, 8 and 12 weeks



Discussion

DISCUSSION

Various temporary anchorage devices have been introduced in recent years for skeletal anchorage, including “onplants”, mini-implants, miniscrews, and microscrew. These devices offer several advantages, such as sufficient anchorage in non-compliant patients, smaller in size, versatility, relatively low cost, ease of insertion and removal. Although these devices are available in several sizes and shapes, the most popular and widely used temporary anchorage devices appears to be the miniscrew.[21]

Possible insertion sites for these miniscrews include the palate, mandibular retro molar area, maxillary tuberosity, anterior nasal spine, mandibular symphysis, and edentulous areas of the alveolar ridge. However the most preferred locations is the interradicular space between the roots of adjacent teeth, and placement of miniscrew in the alveolar process between the teeth is a critical procedure.

According to the “safe zone” map of **Poggio et al** [67], miniscrews with a diameter of 1.5 mm needs at least 3.5 mm of interradicular space.

Schnelle et al [73] determined radiographically that orthodontic alignment increases the number of sites with adequate interradicular bone for placement of these screws. They showed that bone stock for placement of miniscrews was found to exist primarily in the maxillary (mesial to first molars) and mandibular (mesial and distal to first molars) posterior regions and interroot distance must be atleast 4mm for placement of miniscrew

Liou et al [53]stated that although miniscrews are stable they do not remain absolutely stationary throughout orthodontic loading. To prevent miniscrews getting displaced and contacting any vital structures, it is recommended that miniscrew must be placed in a non–tooth bearing area that has no foramen, major nerves, or blood vessel pathways, or in a tooth-bearing area having 2 mm of safety clearance between the miniscrew and dental root.

There are several radiographic studies for miniscrew placement in the interradicular spaces which are 2-Dimentional. These conventional 2-Dimentional radiographic studies fails to assess the angulation changes between the interradicular spaces and do not provide complete view of miniscrew after placement, and this lead to the development of 3-Dimentional surgical guides for placement of miniscrew. [28,38,59]

3-D CBCT study by **Chung et al** [19] have showed that, even when the amount of space between roots was increased by 100%, root contact was still high at 65.7% in both maxilla and mandible, CBCT imaging showed a far higher number of root contact than reported with conventional 2-Dimentional radiographs.

Other causes for root damage are, improper placement of miniscrew,[47] migration of miniscrew after loading, [53,78] axial deviation of miniscrew during insertion,[28] anatomic variation in root form, [37] or tooth contact with miniscrew during orthodontic treatment. [41] Hence it is evident that root contact is highly prevalent when miniscrews are used in interradicular spaces. To assess the repair after root damage many animal studies have been done.

Asscherickx et al [4] showed that healing in beagle dogs takes place approximately 12 weeks after root damage with miniscrew and healing was nearly completed after 20 weeks. However, the study was limited to 6 miniscrews inadvertently placed close to the roots.

Chen et al [17] found higher failure rates when miniscrews contact the root surface during placement. When more inflammation was present, the adjacent roots seemed to experience more resorption. Nevertheless, the lesion was repaired with a narrow zone of mineralized tissue deposited on the root surface, which was likely to be cellular cementum, and was mainly filled with alveolar bone, with the periodontal ligament space being maintained.

Brisceno et al [11] stated that following root contact from miniscrew under favourable conditions (no inflammatory infiltrate or pulpal invasion), healing occurs and it is limited to the cementum or the dentin.

Hembree et al [36] studied on experimental beagle dogs and concluded that, the placement of miniscrew can produce immediate and extensive damage to periodontal structures, no differences in the amounts of damage were evident between the immediate, short and long-term damage. Healing often occurred with cementum around the unloaded miniscrew. Unloaded miniscrew, that remain in contact with roots showed varying degrees of healing.

Renjen et al [71] showed that there was evidence of continuous cementum repair seen along the injured root surface. Only in cases of severe injury with displacement of root fragments was ankylosis with the lamina dura noted.

Even though repair of cementum after intentional miniscrew injury has been studied qualitatively in Beagle dogs by several authors, the results of these studies cannot be extrapolated to the human beings.

In a case report **Maino et al** [54] assessed the repair of root damage from miniscrew in human subjects and his histological results showed that, cellular cementum had been deposited in the damaged area, almost entirely filling the resorption craters within 8 weeks of healing period. In a case series **Kadioglu et al** [41] assessed root repair following miniscrew contact in human samples using SEM and showed that there was repair of damaged root surface with miniscrews and healing period was almost 8 weeks of damage, but the sample size was very small in this study.

Khounganian et al [42] reported that, in order to understand the nature and healing potential of the calcified tissue in non-diseased and diseased root surface, the knowledge of the elemental content of non-diseased and diseased root surface is required, therefore to understand the healing potential of damaged cementum by miniscrew, the knowledge of the various elemental contents and its changes in undamaged area and repaired area is required.

To the best of our knowledge, quantitative study assessing the compositional changes of damaged cementum during repair process have not been performed in humans or in animals after root damage following miniscrew placement.

Therefore the present study was undertaken to quantify the compositional changes of root repair in human subjects following intentional root contact using miniscrew. The ethical consideration in this study was of intentionally inserting miniscrew on the roots of first premolar in clear-cut therapeutic extraction cases. The present study should be comparable to the experimental setup in recent histological and SEM studies on the incidence and repair after application of heavy forces with orthodontic springs to move against compact cortical bone and miniscrews [24, 41,54,65,79,] and in caries studies when premolars were used as an in-vivo cariogenic models. [62]

Root surface damaged by miniscrew intentionally showed various changes depending on the root morphology and diameter of the contacted miniscrew. Since the aim of our study was to quantify the compositional changes occurring during repair process of root surface and not to assess the degree of damage, repair process was confirmed by observing collagen fiber reorganisation in the damaged area by employing scanning electron microscope (SEM) as reported by **Kadioglu et al,**[41] which has magnification ranging from 20X to approximately 30,0000X and to assess the compositional changes in root surface, Energy dispersive X-ray (EDX) analysis was used as reported by **Khounganian et al.**[42]

In our study, teeth were subjected to stereomicroscopic studies to confirm the extent of damaged before subjecting them to SEM studies. In our study, perpendicular placement of miniscrew was employed, the depth of penetration varied at least one fourth to one half of the radius of the miniscrew. Although there were no complaints of abnormal pain or hypersensitivity by patients, there was instance of discomfort and miniscrew used to create defect in our study was not left in place, hence the trauma was transient.

First bicuspid extracted immediately after root contact with miniscrew served as control samples, which was latter compared with the 4th, 8th and 12th week group of the same patient thereby avoiding the inter-individual variation.

Scanning electron microscopic evaluation (SEM):-

Control Group:

SEM microphotographs showed there was clear demarcation between the damaged area and undamaged area. At higher magnification, damaged area showed denuded dentin and cementum surface which was devoid of periodontal ligament compared to adjacent intact periodontal ligament in undamaged area.

Group A:-

SEM microphotographs showed that in the 4th week of repair period there was demarcation between damaged and undamaged area. Stress cracks were well evident at 60X magnification, which was caused due to miniscrew contact. Immature organic collagen fibers were developing, which indicates the early sign of repair process. This result concurs with previous study by **Tronstad et al,** [77] who stated if the cementum is mechanically damaged and the dentin surface is exposed, multinucleated cells will colonize the denuded surfaces, and resorption will takes place. However, the resorbing cells require continuous stimulation during phagocytosis and repair with cementum-like tissue will occur within 2 to 3 weeks of damage.

Group B:-

At the end of 8 weeks of repair, SEM microphotographs showed that stress cracks were still evident and there were more matured collagen fibers in the damaged area, which clearly indicates the regeneration of the periodontal ligament in relation to adjacent undamaged area. This finding agrees with previous study by **Maino et al** [54] who reported that damaged area were almost entirely filling the resorption craters within 8 weeks of healing period.

Group C:-

At the end of 12 weeks of repair, complete regeneration of periodontal ligament takes place and difference between damaged and undamaged area was hardly noticeable and resorption lacunae was almost completely filled with calcified collagen matrix . The amount of repaired process had increased with time.

This finding agrees with the previous study by **Kadioglu et al** [41] who reported that, swift repair of damaged root surface with miniscrews were still discernible at 8th week, but immature fibers organization in the deepest crater represented the ongoing repair process after 8 weeks.

All teeth, inspite of varying degrees of damage, remained vital and without any mobility even after 12 weeks of injury. The SEM findings of this study confirm that a normal repair process was in progress which was characterised by the regeneration of periodontal ligament which was evident by the end of 8th week itself.

The repair process in this study was confined to the periphery of the damaged area and was similar to the findings of **Hellden et al,** [35] who reported that cementum repair process starts from periphery and then proceeds to involve the entire defect.

Since our study was on the surface morphology of root in a non-destructive manner and the damaged area is small, cross section of the damaged area was not done. The undamaged area of cementum surface had homogenous appearance with prominence of extrinsic collagen fibers obscuring the cemental surface, while the damaged surface area were irregular, uneven and devoid of periodontal ligament attachment initially and later on there is reorganization of collagen fibers and regeneration of cementum surface. According **Gonçalves et al,**[33] periodontal ligament is one of the sources of cementoblasts progenitor cells for cementum regeneration.

Energy Dispersive X-ray analysis (EDX) for quantitative assessment:-

To assess the surface compositional changes of repair area, Energy Dispersive X-ray (EDX) analysis was used and its main advantage is it can analyze in the order of a few cubic microns in a non-destructive manner for variety of calcified tissues, such as bone,[56] cartilage,[13] enamel,[30,31] dentin,[8,57] cementum.[45,42,72]

In our study EDX was used to assess the compositional changes that occurring during repair process of damaged root surface and it was compared with the adjacent undamaged area of the same tooth in an area about 1mm around the damaged area. The X –ray intensity counts obtained at each point were converted to percentage with standard magnification of 100X. The elements detected in damaged area and undamaged area on 4th, 8th and 12th week were calcium(Ca), phosphorus(P), sodium(Na), chloride(Cl), carbon (C) and oxygen(O).

Neiders et al [61] found that differences in the mineral composition of cementum from person to person, from tooth to tooth in the same subject and between the various cementum types and his quantitative analysis reported that calcium composition in the cervical is 25.6% and midroot region 26.0% and composition of phosphorus is 12.9% in cervical and 13.5% in midroot region.

Rex et al [72] found mean concentrations of cementum in human first bicuspid and he reported that mean Ca concentration was 27.17% to 30.40%, mean phosphorus concentration was 12.43% to 14.15% and Fluoride concentration was 0.37% to 1.49% there was a decreasing gradient in the Ca and P concentrations from the cervical to the apical third of the root and increasing gradient in the Ca and P concentrations from the outer to inner third of cementum at the cervical and middle thirds of the root. And there was large interindividual variation in Ca, P and F concentration. According to their suggestions and to avoid the error, the elemental composition of undamaged cementum is taken just 1mm around the damaged area.

Our study showed that mean concentration of calcium was 26.88% to 28.33% and mean phosphorus was 13.33% to 13.59% in undamaged and this results agrees with previous quantitative studies of cementum. [20,72,74]

EDX results in Table I showed that mean concentration of elements in repaired area was higher than when compared to adjacent undamaged area in 4th, 8th and 12th week, this difference was statistically insignificant.

These results agrees with previous studies [20,42] as reported that, periodontally diseased cementum had higher concentration when compared to non-diseased cementum this was because when root surface is exposed to oral cavity, there was exchange of minerals at the cementum-saliva interface which resulted in highly mineralized surface zone. Although the teeth were not periodontally diseased in our study during the period of extraction, still it showed hypermineralization.

Kodaka et al [45] reported that, in addition to saliva-cementum interface, hypermineralization can also occur due to blood and tissue fluid interface. **Selvig et al** [74] reported that, if an increased mineral content is observed it is probably due to an increased degree of mineralization.

EDX results (table II) showed that, there was periodically decreasing gradient of calcium and phosphorus concentration, in the ratio between repaired and undamaged area from 4th to 12th week in which calcium was statistically significant and this may be because of initially during 4th week there was hypermineralized surface, later on during the subsequent 8th and 12th week this hypermineralized surface exhibits normal mineralization and for sodium there is increasing gradient of concentration from 4th to 12th week but it was not statistically significant.

This mineralized coating may be derived from components of inflammatory exudate from tissue fluid, which was previously reported by **Eide et al.** [27]

In the present study, the presence of minerals such as sodium, Chloride, Carbon and Oxygen are normal inhabitant of cementum which has not been reported previously.

In the present sample, fluoride concentration had not been detected in the standard magnification of 100X. This agrees with previous report by **Rex et al** [72] who stated that, variability in fluoride concentration between subjects was expected, because they would have been exposed to different levels of fluoride during their lives, and fluoride concentration in cementum are directly related to the amount of ingested fluoride.

The elements detected and its differences in concentration were noted within the root structure could be due to the concentration of these ions in body fluids during tooth formation. It is also possible that the dietary intake and ion exchange after tooth eruption could be reflected within the roots as reported by **Khounganian et al.** [42] More research is needed to see if the difference in concentration of these elements is significant in repair process of damaged root surface by miniscrew.

It is well documented in literature that the incidence of root damage is inevitable even if there is adequate interradicular space. Hence, understanding the exact phenomenon of root repair and compositional changes of damaged area during its repair process after miniscrew placement plays an important role.

These findings indicate that, even though mature collagen fibers are seen at the end of 8^{th} week itself, it takes almost 12 weeks for complete repair. Calcium and phosphorus concentration were initially increased at 4^{th} week but later on in the subsequent 8^{th} and 12^{th} weeks it was found in decreasing gradient of concentration, suggesting that initially hypermineralization occurs in the damaged area in relation to adjacent undamaged area and later on there is a reduction in concentration suggesting that hypermineralized area assumes normal mineralization in relation to adjacent undamaged area.

Clinicians should be aware of iatrogenic damage caused by miniscrews. Further studies with more samples are essential to find out the long term prognosis of damaged root surface caused by miniscrews and compositional changes occurring during the repair process.

Summary and Conclusions

SUMMARY AND CONCLUSION

`This study was done to evaluate the quantitative assessment of root damage and repair following placement of miniscrew using scanning electron microscope and energy dispersive X-ray analysis. The study group consists of 10 patients, five males and five females with a mean age of 16.2 years (13.5 - 21.6 years). Patients warranting first bicuspid extraction for orthodontic treatment were included in the study the sample size was 40 teeth.

With the guidance of intraoral radiographs and wire guide, miniscrews were placed on the distal side of the first bicuspid. After root contact of premolars, the miniscrews were immediately removed and were observed after a repair period of 4, 8, and 12 weeks. After the respective observation time periods the premolars were extracted and assessed using scanning electron microscope (SEM) and elemental compositional changes were analysed by energy dispersive x-ray analyser (EDX). Teeth were subjected to stereomicroscopic studies to confirm the extent of damaged before subjecting them to SEM studies.

Results of our SEM microphotograph showed that, in the 4 weeks of repair, stress cracks were evident, denuded dentin and cementum was covered by calcified matrix, immature organic collagen fibers were developing in the damaged area. And in the 8 weeks repair, immature organic collagen fibers are tends to mature, stress cracks were still evident during this period. The end of

12 weeks of repair, complete regeneration of periodontal ligament takes place and difference between damaged and undamaged area was hardly noticeable and resorption lacunae was almost completely filled with calcified collagen matrix.

Results of our EDX analysis showed, mean concentration of elements in repaired area were hypermineralized that of adjacent undamaged area, further there was periodically decreasing gradient of calcium and phosphorus concentration, in the ratio between repaired and undamaged area from 4th week to 12th week. Later on in the subsequent 8th and 12th week this hypermineralized surface area progresses to normal mineralization.

The conclusions drawn from the study are:-

1) Even though mature collagen fibers are seen at the end of 8th week itself, it takes almost 12 weeks for complete repair of teeth damaged by miniscrew.
2) The mean concentration of elements in repaired area was higher than the adjacent undamaged area in 4th, 8th and 12th week.
3) Initially during 4th week there was hypermineralization in the damaged area, later on during subsequent 8th and 12th week this hypermineralized surface exhibited normal mineralization.

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